THE ROLE OF AUDITORY CORTEX IN SOUND DISCRIMINATION AND PERCEPTION: INSIGHTS FROM OPTOGENETICS

by

CONOR THOMAS O'SULLIVAN

A DISSERTATION

Presented to the Department of Biology
And the Graduate School of the University of Oregon
in partial fulfillment for the requirements
for the degree of
Doctor of Philosophy

September 2019

DISSERTATION APPROVAL PAGE

Student: Conor Thomas O'Sullivan

Title: The Role of Auditory Cortex in Sound Perception and Discrimination: Insights from Optogenetics

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Biology Department by:

Shawn Lockery	Chairperson
Michael Wehr	Advisor
Cris Niell	Core Member
Terry Takahashi	Core Member
Matt Smear	Institutional Representative

and

Janet Woodruff-Borden	Vice Provost and Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded September 2019

DISSERTATION ABSTRACT

Conor Thomas O'Sullivan

Doctor of Philosophy

Department of Biology

September 2019

Title: The Role of Auditory Cortex in Sound Perception and Discrimination: Insights from Optogenetics

The modern era of neuroscience is characterized by massive amounts of data from various methods of recording neuronal activity, but the role of this activity in behavior remains elusive in many cases. Traditional manipulations of activity such as surgical lesions or muscimol application often provide inconclusive information due to their limitations. In this dissertation, I focus on the role of mouse auditory cortex in simple and complex sound discrimination, using PV-ChR2 optogenetic suppression to establish a causal relationship between activity and behavioral performance.

I first examined pure tone discrimination, comparing the effects of optogenetic suppression and electrolytic lesions. Performance was unaffected by lesions but impaired by optogenetic suppression time-locked to stimulus presentation, showing the necessity of auditory cortex activity for successful discrimination. When optogenetic suppression was extended to multiple trials and then the entire lesion recovery duration, the impairment remained, suggesting a fundamental difference between the brain's adaptation to optogenetic suppression and permanent lesion damage.

Optogenetic suppression also impaired phoneme discrimination ability in mice. This effect remained when either the “consonant” or “vowel” segments were temporally targeted for suppression. Finally, I observed potential laser-related learning that could complicate optogenetic suppression analysis, showing that mice can learn to associate random rewards with the laser light cue or adapt to suppression when laser trials are reinforced.

This dissertation includes previously published co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: Conor Thomas O'Sullivan

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene
Princeton University, Princeton

DEGREES AWARDED:

Doctor of Philosophy, Biology, 2019, University of Oregon
Bachelor of Arts, Molecular Biology, 2011, Princeton University

AREAS OF SPECIAL INTEREST:

Neuroscience

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, Department of Biology, University of Oregon, Eugene, 2011- 2019
Undergraduate Researcher, Princeton University Department of Molecular Biology, 2010-2011

PUBLICATIONS:

O'Sullivan C. T., Weible A. P., Wehr M. (2019) Auditory cortex contributes to discrimination of pure tones. In review at *eNeuro.*

O'Sullivan C. T., Weible A. P., Wehr M. (2019) Auditory cortex contributes to discrimination of phonemes. Pending submission to *Frontiers in Neuroscience.*

ACKNOWLEDGMENTS:

First, I would like to thank my PI, Mike Wehr, for all his contributions and guidance throughout my graduate work. He provided a lab environment where I felt empowered to come up with my own ideas and experiments, even if they were outside the traditional topics of the lab. In addition to the general contributions of the members of the Wehr lab, I would especially acknowledge Aldis Weible for his expertise with surgical and electrophysiological techniques, Lucas Ott for countless hours running the mice through behavior, Alexandra Moore for general science ability and a voice that sounds much less weird than mine when pitch-shifted, and Jonny Saunders for coding and analysis advice. Also, these experiments would have never been possible without the contributions of Erik Flister, who provided the behavioral software known as the "Ratrix".

My family has provided curiosity and interest in my project, especially when my own was lacking. Perhaps it was just payback for all the random questions I asked about economics and the world when I was a kid, but thank you Dad for the (usually) insightful questions that forced me to think carefully about my own ideas. Thank you Mom for the support and encouragement that helped me persevere through difficult times.

TABLE OF CONTENTS

Chapter Page

LIST OF FIGURES

Figure Page

CHAPTER II

CHAPTER III

LIST OF TABLES

Table Page

CHAPTER I: INTRODUCTION

Throughout the history of sensory neuroscience, researchers have constantly developed new ways of connecting brain activity to perception. Advances in recording techniques and data processing have generated massive amounts of data, facilitating detailed analysis of how neurons represent information. Recent developments in calcium imaging and multisite electrode recordings have continued this trend and enable recording from many neurons within a population simultaneously (Stosiek et al., 2003; Sakata et al., 2009), but interpretation of the role of this activity remains a challenge.

Using Lesions to Identify Necessity of Activity

Despite extensive characterization of activity patterns, it has remained difficult to determine what is actually necessary for accurate perception and decision making. During any given behavioral task, there is an abundance of activity across the brain that is possibly related to the task demands, but what areas or neurons are actually used or interpreted to make decisions? To establish a causal relationship between activity and behavior, the effects of manipulating or suppressing that activity must be directly tested. Historically, studies using permanent surgical or electrolytic lesions targeted to specific brain areas have been used to show whether performance suffers following damage and interpreted as revealing the necessity of activity in that area (Talwar et al., 2001, Ohl et al., 1999). While this may be a valid interpretation in some cases, compensatory mechanisms or plasticity could be obscuring the true role of targeted areas. If a brain area is normally used for stimulus discrimination but is fully replaced by another pathway or

area following damage, lesion studies would generate results equivalent to a scenario where the targeted area was never involved. There is typically a recovery period for the animal following the lesion due to the disturbance caused by surgery or electric shock, further increasing the opportunity for plasticity before the animal can be tested again to evaluate its behavior. An alternative suppression method, application of muscimol, has the benefit of not being permanent, but it is difficult to control the exact spatial area of its effect as it diffuses through cortex. Additionally, analysis is similarly restricted to comparison of two epochs: before and after muscimol application (Gimenez et al., 2015).

Benefits of Optogenetic Approach

Recent advances in manipulation of neuronal activity have opened a new avenue for investigation that has the potential to resolve this ambiguity. Through the combination of transgenic animals and light-sensitive proteins, optogenetics allows manipulation of specific neuronal populations on millisecond timescales. Crucially, this process is reversible, as neurons quickly return to their normal state following the end of laser stimulation. Rather than being restricted to analysis before and after a lesion, optogenetic techniques allow suppression trials interleaved with control trials during a behavioral task. This reduces potential confounding variables by allowing direct comparisons of performance within the same behavioral session. In addition, the structure of targeted brain areas remains intact, increasing the validity of comparing control and laser trials and reducing the potential for activation of plasticity mechanisms. In several examples,

optogenetic suppression experiments have revealed a role for activity that is not indicated by lesion studies.

In my experiments, I used the specific optogenetic technique of Channelrhodopsin-2 (ChR2) stimulation of parvalbumin(PV)-expressing neurons in transgenic mice. PV neurons are distributed throughout cortex and form local inhibitory connections onto thousands of principal neurons. By activating PV neurons, it is possible to take advantage of the natural inhibitory circuitry to achieve widespread suppression of principal neurons. Alternate strategies such as directly using archaerhodopsin (Arch) expressed in principal neurons for suppression are limited by expression patterns. In that case, only neurons expressing Arch will be suppressed, while all other principal neurons will have normal activity. The PV-ChR2 strategy takes advantage of the high penetrance (~90%) of the PV promoter and the highly connected nature of PV interneurons to reliably achieve nearly total suppression of targeted areas (Moore and Wehr, 2013). Since this technique uses transgenic mice, all PV-expressing cells will have ChR2, so control of spatial extent of suppression in the brain is determined by laser fiber location and power. Prior work in my lab (Weible et al., 2014) has established the spatial extent of potential laser powers, which guided my experimental design.

The Mouse as a Model Organism

As a model organism, mice provide a variety of advantages for examining the link between behavior and neuronal activity. There are bountiful genetic tools available, allowing the use of transgenic mice and removing the need for viral injections of

optogenetic proteins. This leads to more consistent levels of suppression across individual animals by removing the potential randomness and variability involved in the injection and infection process. In addition, mice can be bred quickly to generate large pools of subjects for behavioral experiments. They can be trained via restricted water, only receiving it while performing the discrimination task. Training can be largely automated with behavioral software, only requiring an experimenter to place the mouse in the behavior box, open its file, and then take the mouse out once the session is complete (Saunders and Wehr, 2019). With proper management of water restriction, mice can run hundreds of trials each day, supporting rapid collection of data.

Auditory Cortex

To learn more about the general link between activity and behavior, I focused on the specific example of auditory cortex and sound discrimination. Mouse auditory cortex is tonotopically arranged in a similar manner to humans and other mammals, allowing some generalization of results. Mice can be trained to discriminate both simple and complex (Saunders and Wehr, 2019) sound stimuli. Simpler sounds have the advantage of being easier for mice to learn to discriminate, reducing the time required before optogenetic testing, but have less relation to the complexities involved in human sound perception. Speech perception is of particular interest because it is often impaired as humans age and this deficit can be very detrimental to quality of life (Helfer and Freyman, 2008). Determining the role of auditory cortex in speech discrimination could

help guide further investigation into potential therapies or treatments for age-related deficits in humans.

Prior Studies on Auditory Cortex and Tone Discrimination

Prior lesion studies examining pure tone discrimination have not found significant deficits in performance following destruction of auditory cortex (Ohl et al., 1999, Talwar et al., 2001). Muscimol studies have provided conflicting results, with some evidence for impairment and no effect in other cases (Gimenez et al., 2015; Talwar et al., 2001). The lack of clarity from these results provides an opportunity for insights from optogenetic suppression.

Prior Studies on Auditory Cortex and Phoneme Discrimination

Since phonemes are spectrotemporally complex sound stimuli thought to be processed in auditory cortex, prior work has examined neuronal responses and the effects of lesions. Through the use of a variety of phoneme stimuli, discriminability of auditory cortex neuronal responses, measured by classifier accuracy on pairs of phonemes, has been shown to be correlated with behavioral discrimination accuracy of those pairs (Engineer et al., 2008; Centanni et al., 2013). Using only the first 40ms of responses is sufficient to establish this correlation, but the effect disappears if spike timing information is scrambled, providing further support for the role of auditory cortex in phoneme discrimination. Lesions of rat auditory cortex impair discrimination ability on

phoneme stimuli (Porter et al., 2011), but the effects of optogenetic suppression have not been investigated.

Experimental Design

To investigate the necessity of auditory cortex in tone discrimination, I compared the effects on behavioral performance of optogenetic suppression using the PV-ChR2 strategy and electrolytic lesions. These experiments are described in chapter II and correspond to the first paper submitted for publication, which is co-authored by Aldis Weible and Mike Wehr. Optogenetic suppression impaired behavior while lesions did not, so after initially testing optogenetic suppression exclusively during stimulus presentation, I modified the parameters in order to make the manipulation more analogous to the timing and structure of the lesion procedure. Even in the most similar conditions possible, optogenetic suppression still generated a deficit in performance. This supported the idea that there is a fundamental difference between the brain's response to lesions and optogenetic suppression.

I tested the link between phoneme discrimination ability and auditory cortex activity by using the same PV-ChR2 suppression strategy while mice discriminated between the words "sad" and "dad". These experiments are described in chapter III and correspond to the second paper in the submission process for publication, which is co-authored by Aldis Weible and Mike Wehr. Mice showed a behavioral deficit during laser suppression, with further testing of suppression isolated to the "consonant" or "vowel" segments of the stimuli showing equivalent effects. I also manipulated task reward

structure and laser visibility to observe laser-relating learning, showing that mice can adapt their behavior to some changes in task parameters.

CHAPTER II:
AUDITORY CORTEX CONTRIBUTES TO DISCRIMINATION OF PURE TONES

JOURNAL STYLE INFORMATION: submitted to eNeuro
AUTHOR CONTRIBUTION: Conor T. O'Sullivan, Aldis Weible, Mike Wehr

This chapter is a manuscript that has been submitted to eNeuro. In this paper, I was the primary person responsible for experimental design, data collection/analysis, and the bulk of writing. I received assistance from the second author (Aldis Weible), who carried out and analyzed electrophysiology experiments as well as assisting with surgical procedures. Mike Wehr, the final author, is my lab's PI and assisted throughout the experiment and writing process.

Introduction

Neurons in auditory cortex are well-tuned for frequency, and are organized into multiple tonotopic maps across the cortical surface. Is auditory cortex involved in the perception and discrimination of sound frequencies? For pure tones, across a wide range of species and behavioral paradigms, the consensus view has been that the answer appears to be no. Although there are conflicting results, lesion studies have generally shown that frequency discrimination of pure tones is not affected by ablation of auditory cortex, even after extensive lesions of all known auditory cortical fields (Meyer and Woolsey, 1952; Butler et al., 1957; Thompson, 1960; Goldberg and Neff, 1961; Sellick, 1983; Buser and Imbert, 1992; Ohl et al., 1999; Talwar et al., 2001; Rybalko et al., 2006; Porter et al., 2011; Gimenez et al., 2015). Discrimination of more spectrotemporally complex sounds such as frequency-modulated tones is impaired by lesions of auditory

cortex, suggesting that auditory cortex is recruited when task demands require spectral or temporal integration (Ohl et al., 1999). The effects of transient inactivation on pure tone discrimination (for example, with local muscimol application) have been inconsistent, with some studies reporting no effect while others reporting complete impairment (Talwar et al., 2001; Gimenez et al., 2015). The fact that some transient inactivation studies observed complete impairment suggests that auditory cortex could potentially be involved in frequency discrimination, and that the effects of lesions could differ from those of transient inactivation because of cortical reorganization or some other long-term recovery or compensatory processes. Lesion studies typically include at least several days of recovery after surgery, which could allow time for cortical or subcortical plasticity to eventually allow alternative structures or pathways to mediate frequency discrimination.

More recently, optogenetic suppression experiments in other neural systems have shown that acute suppression can reveal involvement of a brain structure in specific tasks even when lesions of the same structure have no effect (e.g. Goshen et al., 2011; Kumar et al., 2013; Otchy et al., 2015; Hong et al., 2018). For example, remote contextual fear memories are unaffected by hippocampal lesions, but can be abolished by transient optogenetic hippocampal suppression (Goshen et al., 2011). This suggests that a brain structure could be critically involved in specific functions when it is intact and "online," despite the existence of alternative pathways that are adequate for that function. Optogenetic studies such as these are thus providing new insights into redundancy and interactive processing in the brain, aspects which can be both evolutionarily adaptive and experimentally vexing. Such findings also prompt a re-evaluation of conclusions that

structures (such as auditory cortex) are not involved in a task (such as frequency discrimination) based on lesions that produced no deficit in task performance.

Here we compared the effects of lesions and optogenetic suppression of auditory cortex on frequency discrimination in mice. For suppression, we used mice that expressed Channelrhodopsin2 in parvalbumin-expressing interneurons (PV-ChR2), and trained them in an operant task to discriminate the frequency of brief pure tones for a water reward. We found that transient bilateral optogenetic suppression partially but significantly impaired discrimination performance. In contrast, bilateral electrolytic lesions of auditory cortex had no effect on performance of the identical task, even when tested only 4 hours after lesion. This suggests that when auditory cortex is destroyed, an alternative pathway is adequate for mediating frequency discrimination. Yet this alternative pathway is insufficient for task performance when auditory cortex is intact but has its activity suppressed. These results indicate a fundamental difference between the effects of brain lesions and optogenetic suppression, and suggest the existence of a rapid compensatory process possibly induced by injury.

Methods

Mice

For optogenetic suppression of auditory cortex, we used mice that were offspring from a cross of homozygous Pvalb-IRES-Cre ("PV", 008069; The Jackson Laboratory) and homozygous CAG-ChR2-eYFP ("ChR2", 012569; The Jackson Laboratory) lines, which are on a C57Bl6/J background (n = 19 mice in total). In these mice ("PV-ChR2"),

ChR2 was expressed in parvalbumin-expressing (PV+) interneurons, with 97% specificity in auditory cortex (Moore and Wehr, 2013). We used C57Bl6/J mice that did not express ChR2 as controls (n = 5 mice; 3 were -/ChR2, 1 was -/-, 1 was GPR26-cre/-). For electrolytic lesion experiments, we used wild-type C57Bl6/J mice (n = 5 mice).

Surgical procedures

We administered dexamethasone (0.1 mg/kg) and atropine (0.03 mg/kg) pre-surgically to reduce inflammation and respiratory irregularities. Surgical anesthesia was maintained with isoflurane (1.25-2.0%). For optogenetic manipulation, we implanted 200 μm optic fibers in each hemisphere at AP ~2.3 mm (relative to bregma), ML 4.4 mm, and depth 0.5 mm below the dura (just dorsal to primary auditory cortex). The implants were painted with black acrylic paint to minimize light leakage. For electrophysiological verification of optogenetic suppression, we implanted 2 mice (not used in behavioral experiments) with a unilateral optrode array, consisting of 8 tetrodes and a 200 μm fiber terminating 1 mm above the recording sites. The 8 tetrodes passed through two 28-gauge stainless steel hypodermic tubes, with 4 tetrodes per tube. The optic fiber was fixed in position immediately adjacent to, and between, these tubes. Tetrodes were made of 18 μm (25 μm coated) tungsten wire (California Fine Wire). The entire array was mounted on a custom microdrive. The optrode array was inserted vertically through a small craniotomy (2 mm x 1 mm) dorsal to auditory cortex, and cemented into place. For electrolytic lesions, we implanted a pair of stainless steel wires (112 μm diameter, 150 μm coated) spaced ~750 μm apart into auditory cortex in each hemisphere. The teflon coating was

stripped 500 μm from the tips. Pairs were implanted at ML 4.2 mm, DV: 1.0 mm, centered on AP: -2.9 mm. We administered ketoprofen (4.0 mg/kg) post-operatively to minimize discomfort. Mice were housed individually following the surgery and were allowed 7 days of post-operative recovery.

Histology

Brains of mice used for electrophysiological validation were sectioned (100 μm) in the coronal plane to verify the position of single neuron recording sites. Only data corresponding to tracks located within auditory cortex were included.

Behavioral Setup

Mice performed the task in sound-attenuating behavioral chambers. Within the chamber, mice were placed in a plastic arena, one wall of which contained 3 combination ports for lick-sensing and water delivery. Each port had an IR beam-break sensor, at which mice responded by licking, and a tube to deliver water rewards for correct responses. Sound stimuli were controlled by a computer running custom behavioral software (modified from Meier et al., 2011), and delivered through 2 speakers placed outside the arena facing the ports. Since laser illumination was delivered with blue light that could potentially be visible to the mouse, we used a color-matched blue strobe light (full-field illumination at approximately 10 Hz) to mask laser stimulation. Mice were trained for an hour each day for 5-7 days a week, corresponding to 300-500 trials and 1-2 g of water reward per day.

Table 1: Training steps - Optogenetic Suppression

Training Step	Description	Advancement criterion
1. Surgery	Fiber implantation	1 day of water restriction post-recovery
2. Free drinks	Ports give water, no stimulus	Trial rate (cannot trigger the same port repeatedly)
3. Requests rewarded	Rewards given for center-port trial requests and for correct responses	Trial rate (7 trials in 30 s)
4. Only correct responses rewarded	Rewards only given for correct responses	400 trials completed
5. Longer penalty	Increased timeout for incorrect responses, no correction trials	Sustained performance > ~85%
6. Optogenetic suppression	Laser on for 10% of trials	N/A

Prior to any behavioral training, the mice underwent surgical implantation of optical fibers or lesion electrodes (step 1, see Table 1). Mice started training with a simple lick-for-water task to familiarize them with the operation of the ports in the absence of sound stimuli (step 2, “Free drinks”). Next, they advanced to the first stage of the main task (step 3). In the main task, mice requested trials by licking the center port, which triggered stimulus delivery. Mice responded by licking at the left port (for 4 kHz, 500 ms pure tones) or the right port (for 13 kHz, 500 ms pure tones). Correct responses triggered an 80 µl water reward followed by a 1 second delay before the next trial could be

requested. Incorrect responses gave no water and produced an additional 1 second penalty timeout before the next trial. To increase the number of trials performed, some mice had their water rewards reduced to 40 or 60 µl. During an initial shaping stage of the main task (step 3), mice received water rewards at the center port for requesting trials (as well as for correct answers at the side ports) until reaching a rate of 7 completed trials in 30 seconds. Once the mice achieved this rate of trials, the rewards for requesting at the center port were removed (step 4). In steps 3 and 4, we included "correction trials" to reduce the development of response bias to one side or the other. After an incorrect response, there was a 50% chance that a mouse would go into a correction trial sequence, in which the same stimulus was repeated until the mouse responded correctly. Correction trials provide contextual information that could conceivably allow a task strategy that did not depend solely on stimulus discrimination, so we disabled correction trials during the final training stage. After 400 trials at step 4, the penalty timeout for incorrect responses was increased to 3 s and correction trials were turned off (step 5). When mice were performing at 85% or higher on step 5 for approximately 5 days, they were run for at least 2 days with fibers attached to the ferrules on their head but without light delivery, to allow the mice to become accustomed to the tethers. Then mice advanced to the final stage (step 6) for optogenetic suppression experiments, using one or more of the illumination protocols described below.

Stimuli were 500 ms pure tones at two frequencies, 4 kHz and 13 kHz. Sound levels were not identical for all behavior boxes (range: 73 - 82 dB SPL, mean: 77 dB, S.D. 3 dB) but within a given box the sound levels were similar for the two frequencies

(mean difference: 0.3 dB) and were consistent from day to day. Reaction times were measured from tone onset to response port entry, with video frame resolution (16.6ms).

Optogenetic Suppression

In order to suppress auditory cortex, 445 nm wavelength (blue) diode laser pulses were delivered to the implanted bilateral optical fibers, with an output power of 9.5 mW as measured at the fiber tip (corresponding to an irradiance of 300 mW/mm^2). In a previous study using identical fiber implantation and lasers, we electrophysiologically characterized the spatial extent of cortical suppression, which was 1750 μm at this power level (Weible et al., 2014). This extent includes all tonotopic fields of auditory cortex, throughout the cortical depth, but does not include thalamic, collicular, or other subcortical regions. In a subset of mice we also tested a power level of 6.3 mW (200 mW/ mm^2), which has a spatial extent of 1500 μm. We used three different temporal patterns of light delivery, which we refer to as transient suppression, cycle suppression, and sustained suppression.

Transient Suppression

On 10% of the trials, the laser was turned on for the full 500 ms duration of the stimulus (Fig. 1a). Whether the laser would be turned on was decided randomly for each trial with a 10% probability. Laser trials were randomly rewarded in order to avoid the potential learning of new stimulus-reward associations. To control for the possibility of non-optogenetic effects of laser illumination on behavior, we also used identical fiber

implantation and illumination with wild-type mice (n = 5 mice). Although we minimized light leakage with tight fiber connections and black paint on implants, it is conceivable that stray light from laser illumination could be distracting to the mouse, which could be a confounding effect with the intended suppression of auditory cortex. Using non-ChR2-expressing control mice isolates the effect of this potential distractor.

Cycle Suppression

To investigate the effects of prolonged suppression of auditory cortex, we used a cyclic pattern of laser activation, alternating between sets of 20 normal and 20 suppressed trials. For this cyclic laser suppression, we used 100 Hz pulsed illumination trains (5 ms on, 5 ms off) instead of stimulus-locked 500 ms laser pulses (Fig. 3a). Output power during each 5 ms pulse was 9.5 mW. For each cycle, illumination started with stimulus request on trial 1, and ended with response selection on trial 20. Because illumination was sustained for a fixed number of trials, the actual duration of illumination depended on trial rate. In this "Cycle suppression" condition, all trials were rewarded normally (according to the tone frequency), since laser trials made up 50% of the total and we were concerned that this proportion of random rewards might disrupt overall performance.

Sustained Suppression

Sustained optogenetic suppression experiments were designed to mimic the effects of electrolytic lesions. Mice were tested for a half-hour session to provide a same-day baseline measure of performance, then received 4 hours of sustained optogenetic

suppression in a holding cage within the sound-attenuating behavioral chamber, using continuously pulsed 100 Hz laser illumination (5 ms on, 5 ms off, as in cycle suppression). Then, with the continuously pulsed 100 Hz laser illumination still on, mice ran for a second half-hour session. Mice remained connected to optic fibers during the entire period of sustained suppression (i.e. they were not disconnected during transfer to or from the holding cage).

Single Neuron Recording and Analysis

Tetrode data were acquired with 32-channel RHD2000 hardware (Intan Technologies) and Open Ephys software (Siegle et al., 2017). A minimum threshold of 60 μV was set for collection of spiking activity. Activity of individual neurons was isolated offline using MClust (Redish, 2008). Measures of peak and trough waveform voltage, energy, and principal components analysis were used as waveform separation parameters in 2-dimensional cluster space. Cells were accepted for analysis only if they had a cluster boundary completely separate from adjacent cluster boundaries, and completely above threshold, on at least one 2-D view. Cluster boundaries were then applied across sessions to track single cell responses across different stimulus contingencies.

We recorded neuronal responses to pure tones (500 ms duration, 500 ms inter-trial interval, 50 repetitions) with or without transient optogenetic suppression on interleaved trials, as well as spontaneous activity in silence before, during and after sustained optogenetic suppression (100 Hz, 100 second duration, 100 second inter-trial interval, 10 repetitions). Laser power was 6.3 mW, corresponding to an irradiance of 200 mW/mm^2 as

measured at the tip of the 200 µm diameter fiber. All data were collected as mice freely explored a cylindrical plastic container (height 16 cm, diameter 16 cm) inside a double-walled sound-attenuating chamber. Sounds were delivered from a free-field speaker directly above the cylinder. The speaker was calibrated to within ±1 dB using a Brüel and Kjær 4939 1/4-inch microphone positioned within the cylinder approximately at head height. Following each recording session, the tetrode array was lowered ~80 µm and allowed to settle for a minimum of 3 hours before initiating another session to ensure that responses collected during each session reflected the activity of a unique population of cells. Optimal pure tone frequencies were selected by first assessing frequency tuning of individual neurons (4-64 kHz presented at 20, 40, 60, and 80 dB SPL). Significantly tone-responsive cells were identified by comparing the firing rate during the first 75 ms following tone onset to an equivalent period during silence (using the paired t-test). We determined best stimulus (frequency and intensity) from the highest firing rate response across all frequencies and intensities. Because multiple neurons were recorded simultaneously (with 8 tetrodes), we selected best stimuli for several representative neurons and then presented those stimuli with and without illumination to test the effects of optogenetic suppression. After off-line spike sorting, for each cell we chose the single best frequency of those presented and tested the effects of optogenetic suppression by comparing laser-off tone responses to interleaved laser-on responses (paired t-test, entire 500 ms tone duration). Recordings from putative PV cells, as identified by significant firing rate increases during laser pulses in silence compared with an equivalent period of silence with laser off (paired t-test), were excluded from group analyses. To assess the

effects of sustained optogenetic suppression, we compared the mean firing rate during the 100 second pulse train to that for the 10 seconds preceding and 10 seconds following the pulse train (ANOVA). We also compared the first and last 10 seconds of the 100 second pulse train to determine whether suppression was stable over time (paired t-test).

Electrolytic Lesions

To produce bilateral lesions of auditory cortex similar to those achieved with surgical ablations in previous studies, we passed current through implanted electrodes. For these experiments, mice were first implanted with electrodes as described above. Then, after at least 7 days of recovery, mice were trained on the task with steps 1-5 as described above (see Table 1). Mice continued running in Step 5 after performance reached asymptotic maximum, in order to provide several days of pre-lesion baseline data. On the day of the lesion, mice performed a half session (30 min) in the morning to establish same-day baseline, and were lesioned immediately after this session ended. Lesions used 45 s of radio frequency current (~5 W) passed through the bipolar electrodes in each hemisphere. Mice then ran another half session in the afternoon after a median 4 hours of recovery. This approach minimizes the time following the lesion (compared to the days-to-weeks recovery required after surgical ablation), providing the soonest possible testing of post-lesion performance. Mice were anesthetized with 1.5% isoflurane for ~2 minutes during electrolytic lesioning. We noted that mice were disoriented for 1-2 hours after lesioning, so we waited until they were alert and responsive before post-lesion testing (median 4 hours). Following post-lesion behavioral

data collection, we sectioned the lesioned brains and examined them to determine lesion extent. Using coronal mouse atlas sections (Paxinos and Franklin, 2019), we identified auditory cortex via landmarks such as the hippocampus and rhinal fissure and visually inspected sections spanning auditory cortex. We quantified lesion extent as the damaged fraction of total auditory cortex area across sections.

Statistical Analysis

We calculated performance separately for responses on laser and control trials. For individual mice, we measured accuracy (in percent correct) using Fisher's exact test on the contingency tables created by the two stimuli and two possible responses. We limited analysis to the first 10,000 total trials after the start of laser stimulation, to avoid any long-term learning effects. To examine performance as a function of time, we measured performance using a 100-trial sliding window. We tested for group effects using a one-tailed Wilcoxon signed-rank test on the control and laser performance data. We compared performance of electrolytic lesion mice before and after the lesion using the one-tailed Wilcoxon signed-rank test, comparing the performance on the final 700 pre-lesion trials to the first 700 post-lesion trials (which could span sessions). For both of these tests, the group data could be approximated as a normal distribution based on the Lilliefors' goodness-of-fit test, so we estimated effect size using Cohen's d, $d = \frac{z}{\sqrt{n}}$, where z is the z-score and n is the number of mice. To determine the rightward or leftward response bias of each mouse, we calculated the difference between the proportion of rightward responses and rightward stimuli, in sliding 100-trial windows. A difference of

zero indicates no bias, as the choices of the mouse are proportionate to the stimuli presented. Positive values indicate that the mouse is biased to the right, and negative values indicate a bias toward the left. For display, we added 50% to the bias values, such that 50% indicates no bias, values <50% indicate leftward bias, and values >50% indicate rightward bias.

For receiver-operating-characteristic (ROC) analysis, we evaluated performance separately for each stimulus value and then compared between laser and control trials. To reveal stimulus-related effects, we separated each mouse's performance into "13 kHz hit rate" (percent correct on 13 kHz trials) and "13 kHz false alarm rate" (100 - percent correct on 4 kHz trials). This arbitrary assignment to hits and false alarms allows ROC analysis of the effects of laser on both performance and bias. We plotted hits against false alarms, separately for laser and control conditions for each mouse. The distance between these points indicates the magnitude of the laser effect on performance, and the direction between them indicates the degree of induced bias.

Results

Transient Full Stimulus Suppression Impairs Tone Discrimination

We first tested whether transient optogenetic suppression of auditory cortex during tone presentation would impair tone discrimination. We implanted PV-ChR2 mice with optical fibers bilaterally over auditory cortex, and trained them to discriminate between two tones well-separated in frequency (4 kHz and 13 kHz, 500 ms, ~77 dB) to set up for optogenetic suppression during tone presentation (Fig. 1a).To verify that our

optogenetic method effectively suppressed cortical activity, we recorded from auditory cortical neurons in separate mice (not used for behavior) using a tetrode array, attached to an optical fiber implanted in the same location as in the mice used for behavior. We recorded from 90 neurons, 46 of which responded significantly to 500 ms pure tones. We excluded PV cells, which were unambiguously identified by robust responses to illumination (Moore and Wehr, 2013). Figure 1b shows the effect of transient optogenetic suppression (at 200 mW/mm^2) on the response to best-frequency tones and on spontaneous activity. Suppression was nearly complete. Across the population, suppression reduced the mean firing rate during the tone from 10.4 ± 7.2 to 1.5 ± 3.9 Hz (mean ± s.d., $t = 9.7$, $p < 0.0001$, $n = 46$ tone-responsive neurons), and reduced spontaneous activity from 2.3 ± 2.4 to 0.3 ± 1.5 ($t = 9.2$, $p < 0.0001$, $n = 90$ neurons). Tone responses were significantly suppressed by illumination in 44/46 (96%) of tone-responsive neurons, and spontaneous activity was significantly suppressed in 52/90 (58%) of all recorded neurons. Note that off-responses (i.e., responses evoked by tone offset) were unaffected by suppression, because illumination ended at tone offset.

Mice achieved a high level of performance (>85%) within 2,000-4,000 trials (Fig. 1c), after which we illuminated auditory cortex just during the 500 ms tone presentation on 10% of trials (Fig. 1a, 9.5 mW or 300 mW/mm^2). Illumination trials were randomly rewarded to minimize the possibility that mice could learn new stimulus-response associations. Optogenetic suppression significantly, but incompletely, impaired task performance. Figure 1d shows an example of performance for an individual mouse over the first 10,000 trials after the onset of suppression trials. For this mouse, performance on

laser trials was impaired by 10-15% compared to control trials. This was true for all mice, with performance of 90 ± 4.6% on controls trials compared to 75 ± 7.5% on laser trials (Fig. 1e, $p = 0.0019$, one-tailed Wilcoxon signed-rank, effect size $d = 0.63$, $n = 9$ mice). All mice showed significant effects (Fisher's exact test, $p < 0.05$), with individual effects on performance ranging from 10-25% (Fig. 1e). These results show that when auditory cortex is intact and operational, mice rely at least in part on it to perform tone discrimination.

The mouse shown in Figure 1d did not show a strong bias on laser trials, that is, it showed roughly equal numbers of both leftward and rightward responses (grey line in Fig. 1d). However, some mice did develop a bias on laser trials, which could reflect a default strategy when a mouse is uncertain of the correct response. To examine the joint effects of suppression on both accuracy and bias, we turned to ROC analysis. In Figure 1f, the performance of each mouse on control trials is represented by an open symbol, and on laser trials by a cyan symbol. Performance on control trials is clustered at the upper left corner, indicating high accuracy and low bias. On laser trials, performance for all mice shifted towards the dashed line (chance performance). Bias is indicated by a deviation in the direction parallel to the dashed line (i.e., towards the lower-left or upper-right corners). A shift perpendicular to the dashed line (i.e., towards the lower-right corner) indicates an effect on accuracy with an absence of bias. While all mice showed significant effects on accuracy, most showed little bias, and two mice showed stronger bias (one in each direction).

We used two approaches to minimize the possibility that laser illumination could act as a visual cue that could affect behavior. First, we used black paint to minimize stray light from the optical fibers. Second, we used a color-matched continuous full-field strobe to mask any possible visual stimulation from the lasers (for example, due to intracranial retinal stimulation). To further control for the possibility that laser illumination could act as a visual cue that could affect behavior, we repeated the experiment with non-ChR2-expressing mice. We first used a "normal reward" condition, in which laser trials were rewarded normally according to the appropriate response and the only distinguishing feature of laser trials was illumination. Illumination had no effect on task performance (Fig. 1g, $p = 0.094$, one-tailed Wilcoxon signed-rank, $n = 5$ mice), with individual effects on performance ranging from a 3.1% decrease to a 0.9% increase. We then used random rewards on laser trials, to exactly replicate the conditions used with ChR2-expressing mice. Illumination again had no effect on performance (Fig. 1h, $p = 0.063$, one-tailed Wilcoxon signed-rank, $n = 5$ mice), with individual effects on performance ranging from a 3.5% decrease to a 0.2% increase. From this we conclude that laser illumination of the brain had no effect on task performance in mice not expressing ChR2, and thus that the effects we observed in ChR2-expressing mice were specifically due to optogenetic suppression of auditory cortex.

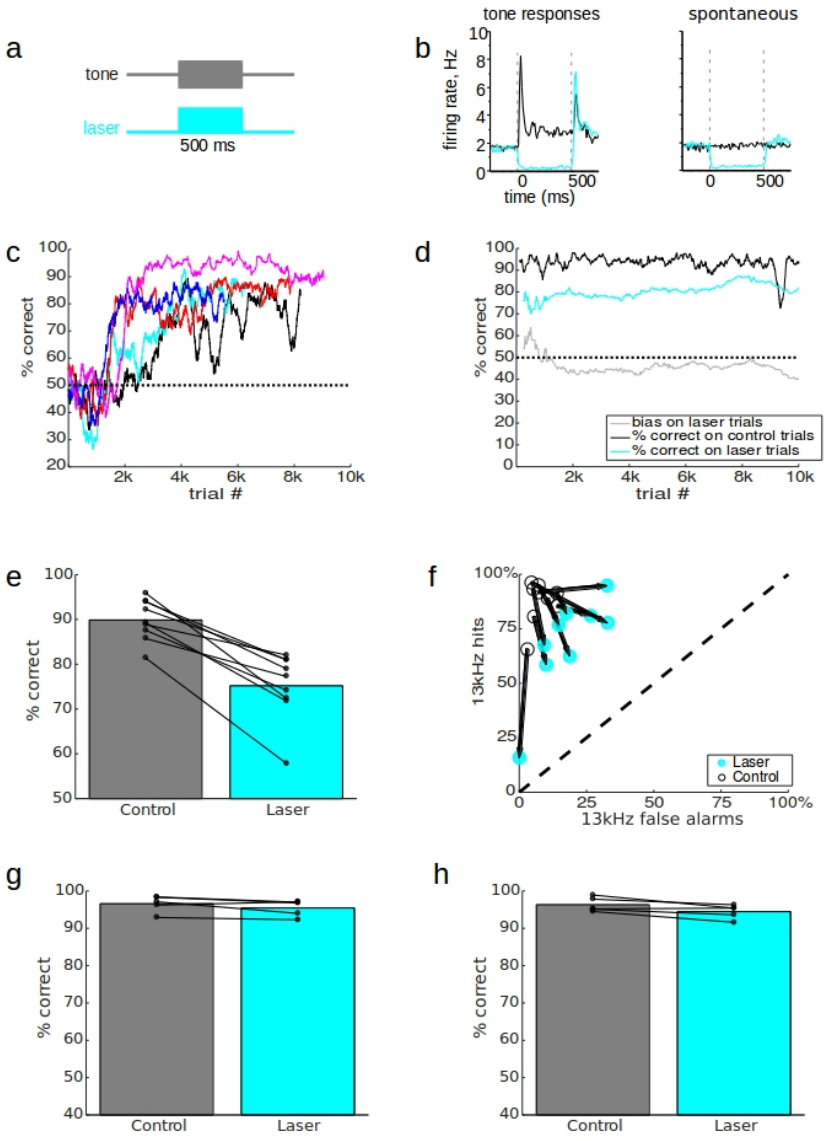
To address potential concerns that laser suppression could reach beyond auditory cortex, we tested a separate cohort of 6 mice on the original 300 mW/mm^2 laser power as well as several additional sessions (1200-3200 trials) at 200 mW/mm^2 (6.3 mW total power), which has a reduced spatial extent (Weible et al., 2014). This laser power also

produced a performance deficit ($p = 0.018$, one-tailed Wilcoxon signed-rank, $n = 6$ mice, effect size $d = 0.86$). The deficits on suppression trials were not significantly different between the two laser powers ($p = 0.22$, one-tailed Wilcoxon signed-rank, $n = 6$ mice), suggesting that deficits can be attributed to effects on auditory cortex.

Figure 1 (next page). Transient optogenetic suppression of auditory cortex impaired pure tone discrimination.
a. Transient laser suppression used a 500 ms constant laser pulse at 300 mW/mm^2 (9.5 mW total power) that completely overlapped with the 500 ms pure tone stimulus. Laser was delivered on a random 10% subset of trials.
b. Electrophysiological validation of optogenetic suppression in separate mice. Left: responses to best-frequency tones averaged across 46 tone-responsive neurons and 50 repetitions under control conditions (black) and during a 500 ms constant laser pulse at 200 mW/mm^2 (cyan). Right: effect of illumination on spontaneous activity during silence, averaged across 90 neurons and 50 repetitions. Both tone responses and spontaneous activity were significantly suppressed.
c. Progression through training steps is shown for 5 example mice, aligned to the start of training. Performance was computed in a 200 trial sliding window.
d. The first 10,000 total trials in the suppression condition (step 6) for an example mouse (mouse rt.15) show a consistent effect of the laser, with minimal effects on bias. A 200 trial sliding window was used to separately compute accuracy over time on control trials (black) and laser trials (cyan). Since this would require ~2000 total trials before having a sufficient sample to measure laser performance, we used a smaller window of 50 trials to measure performance for the initial 100 laser trials, and a 200 trial window for subsequent laser trials. We also measured the bias on laser trials (gray), with 50% corresponding to equal left and right responses.
e. Performance in overall percent correct for each mouse for 10,000 total trials is shown by connected dots for control and laser trials. Bars represent mean performance for each trial type across all 9 mice.
f. To show stimulus-related effects, we used ROC analysis to separate each mouse's performance into "hit rate" (percent correct on 13 kHz trials) and "false alarm rate" (100 - percent correct on 4 kHz trials). The dashed line represents chance performance (50% correct), ranging from 100% leftward (4 kHz) responses at the lower left corner to 100% rightward (13 kHz) responses at the upper right corner. Performance for each mouse on

control trials is represented with a large open circle, connected by an arrow to the laser performance for the same mouse (cyan circle). Perfectly accurate behavior corresponds to the upper left corner, and any effect of the laser towards the dashed line indicates a decrease in performance, which is seen for all mice. Most mice showed little response bias, corresponding to the direction towards the lower right corner (perpendicular to the dashed line), but two mice showed stronger laser effects on response bias (one towards the lower left corner, and one toward the upper right corner).

g-h. Mice that did not express ChR2 showed no significant effects of laser illumination. Format as in e, 5 mice, 2,000 - 4,000 total trials. Rewards on laser trials were either delivered randomly (h), or normally (g; i.e., for the correct response corresponding to the stimulus).



Electrolytic Lesions Do Not Affect Tone Discrimination

The fact that transient optogenetic suppression of auditory cortex impaired tone discrimination performance is surprising, because permanent lesions of auditory cortex have no effect on pure tone discrimination (Butler et al., 1957; Goldberg and Neff, 1961; Ohl et al., 1999). Typical lesion studies require a recovery period of days or longer after surgery, and we wondered whether long-term plasticity or reorganization during this recovery period could contribute to the marked difference between the effects of lesions and transient optogenetic suppression. To address this, we implanted electrodes for producing electrolytic lesions of auditory cortex, and trained mice on tone discrimination after recovery. This allowed us to lesion auditory cortex bilaterally and then test the effects on tone discrimination within a few hours. Lesions were extensive, averaging 56.8 ± 9.3% of AC and 69.3 ± 10.0% of A1 (Fig. 2a). After mice reached stable asymptotic pre-lesion performance, we ran them for a half hour session in the morning, delivered electrolytic lesions, and ran them again the same day after recovery (median recovery time 4 hours). Mice continued to run the task on subsequent days. Lesions had no effect on tone discrimination performance, either in individual mice or at the group level (Fig. 2b-d). Mean pre-lesion performance was 96.3 ± 1.5% and post-lesion performance was 95.7 ± 1.5% (Fig 2d, $p = 0.45$, one-tailed Wilcoxon signed-rank, effect size $d = 0.06$, $n = 5$ mice). Since there was variation in lesion extent, we tested whether this was correlated with behavioral effects (Fig. 2e) and found that it was not (Spearman's correlation, $r = 0.6$, $p = 0.35$). ROC analysis showed no effect of lesions on accuracy or bias (Fig. 2f). These results show that when auditory cortex has been extensively damaged, within 4

hours mice are able to use alternative circuits to perform tone discrimination with no measurable deficit.

In some tasks, overtraining has been shown to shift task dependence from cortex to striatum as performance becomes habitual (Smith and Graybiel, 2013). We therefore wondered whether different amounts of training time could have contributed to the differences we observed between the effects of optogenetic suppression and lesions. However, there was no difference between groups in the number of training trials prior to the manipulation, either for all training (steps 1-5, $p = 0.89$, rank-sum) or for the final stage of training (step 5, $p = 0.61$). Thus groups had no systematic differences in overtraining that could explain the different effects of optogenetic suppression and lesions.

Figure 2 (next page). Electrolytic lesions of auditory cortex did not impair pure tone discrimination.

a. The section of maximal lesion extent for each mouse (n = 5) is shown in cyan/blue, overlaid onto the boundaries of auditory cortex (red).

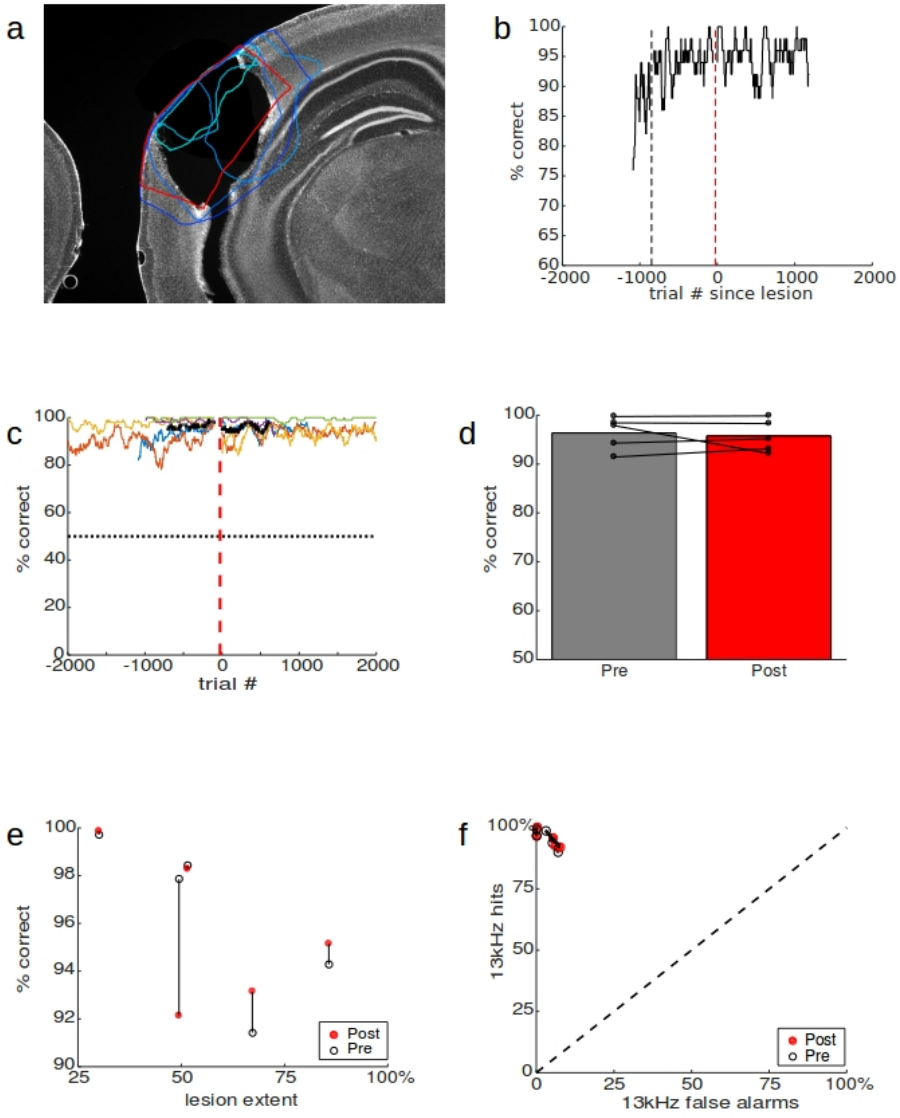
b. Performance before and after lesion for an example mouse (mouse 6685). Vertical black dashed line indicates graduation to the final training step (6). In the 700 pre-lesion trials, overall performance was 94.25% correct. The lesion is indicated by the red dashed line at trial zero. In the 700 trials following the lesion, the mouse performed at 95.1%, showing a lack of impairment. Performance is computed in a 100 trial sliding window. Chance performance is 50%.

c. Performance for each of 5 mice is shown in different colors. Format as in b. Traces are aligned to the time of the lesion, indicated by the red dashed line. Note that a median of 4 hours elapsed at the time of lesion (red dashed line). Heavy black line shows mean across mice for 700 trials before and after lesion.

d. Mean performance across all mice on the 700 trials pre-lesion and 700 trials post-lesion are represented as bars, with the performance of individual mice indicated by connected dots.

e. Lesion extent for individual mice is plotted against their pre-lesion performance (black) and post-lesion performance (red).

f. ROC analysis (same format as Figure 1f). Open circles indicate pre-lesion performance for each mouse, which are connected to red circles indicating post-lesion performance. Lesions had negligible effects on accuracy or response bias.



Cycle Suppression Shows a Sustained Deficit

One interpretation of the lesion and transient suppression experiments described above is that together they provide an upper and lower bound on the time scale required for mice to recover the ability to discriminate tones when auditory cortex becomes unavailable. That is, transient (500 ms) unavailability of auditory cortex produces a deficit, but within about 4 hours some recovery process allows accurate performance even without auditory cortex. The time course for this recovery process must then lie between 500 ms and ~4 hours. To better understand this putative recovery process, we set out to characterize its time course. Because any given trial response is either correct or incorrect, estimating performance accuracy requires integration across trials. For example, at least 100 trials are required to estimate performance (in percent correct) with a precision of 1%. Because our mice perform about 5 trials/minute, 100 trials takes 20 minutes on average. This means that the temporal precision achieved by integrating consecutive trials is insufficient to resolve the fine time scale of the recovery process. We therefore designed a cyclical suppression paradigm so that we could average performance over large numbers of trials with high temporal precision (Fig. 3a). To verify that cycle suppression effectively suppressed cortical activity, we tested this method on a population of 153 auditory cortical neurons recorded with tetrodes (including the cells shown in Fig. 1h, and excluding PV cells). Because tetrode-implanted mice did not perform the task, instead of 20 trials of continuous suppression, we used a fixed duration of 100 seconds (corresponding to the approximate duration of 20 trials, see Fig. 3e). Across the population, suppression reduced spontaneous activity from 1.8 ± 1.9 to 0.9 ± 1.4 Hz

(Figure 3b, $t = 9.2$, $p < 0.0001$, $n = 153$ neurons). Spontaneous activity was significantly suppressed in 102/153 (67%) of neurons. Suppression was stable throughout the 100 second suppression period, with no mean difference between the first 10 seconds of suppression and the final 10 seconds ($p = 0.60$).

After a new group of 10 fiber-implanted PV-ChR2 mice reached asymptotic performance on step 5, we alternated 20-trial blocks of no illumination with 20-trial blocks of 100 Hz illumination. Figure 3c shows the performance of individual mice as well as the group mean averaged across a minimum of 119 blocks, representing at least 19 days of behavior. On trial 1 (the first illumination trial in the cycle), laser onset coincided with tone onset, making it analogous to the laser trials in the 500 ms transient suppression experiment, producing a 12% drop in performance ($p = 0.00098$, one-tailed Wilcoxon signed-rank). Already by the second trial in the cycle, performance recovered substantially. However, performance did not recover to pre-suppression levels by the end of the cycle (comparison of last suppression trial with last pre-suppression trial: $p = 0.002$, one-tailed Wilcoxon signed-rank). Reaction time was unaffected by optogenetic suppression (Fig. 3d) regardless of position within the trial cycle (comparison of first suppression trial with last pre-suppression trial, $p = 0.422$, one-tailed Wilcoxon signed-rank). Mice completed the 20-trial cycles in about 1-3 minutes, which did not differ between laser-on and laser-off blocks (Fig. 3e, $p = 0.461$, one-tailed Wilcoxon signed-rank). This indicates that prolonged suppression produces an immediate deficit followed by a rapid but partial recovery, as well as a sustained deficit lasting at least 20 trials or

about 1-3 minutes. Recovery was not well fit by either single or double exponentials, either with respect to trials or to elapsed time, suggesting a complex time course.

After mice completed at least 19 days of testing with the 20-trial cycle protocol, we tested them with transient 500 ms suppression to compare the strength of effects. Mice showed individual differences in effect sizes, but across mice the effect of transient 500 ms suppression was highly correlated with the effect of cycle suppression (Fig. 3f, $R^2 = 0.7834$, $p = 0.0007$, linear regression, $n = 10$ mice). The effect size for the initial trial of cycle suppression was half of that for transient 500 ms suppression (regression slope $m = 0.502$, y-intercept = 2.5%). This could be due to the 50% duty cycle of the sustained 100 Hz pulse train (5 ms on, 5 ms off).

We wondered whether the incomplete recovery seen after 20 trials of suppression (Fig. 3c) indicated the existence of a longer-lasting recovery process. We therefore extended this cycle-based approach to use cycles of 200 trials with laser on, alternating with 200 trials with laser off. The initial laser onset deficit remained, as seen from a comparison of performance on the final 10 pre-suppression and first 10 post-suppression trials ($p = 0.031$, one-tailed Wilcoxon signed-rank). Mice showed a sustained performance deficit even after 200 trials (Figure 3g, $n = 5$ mice). The deficit in the last 10 trials was no different from that in the first 10 trials ($p = 0.31$, one-tailed Wilcoxon signed rank), nor was it different from the last 10 trials using the 20-trial cycle protocol ($p = 0.5$, one-tailed Wilcoxon signed rank). There was also no trend towards recovery over the course of the 200 trials (linear regression slope $m = -0.032$, $p = 0.686$). Mice completed the 200-trial cycles in a median (across mice) of 20.5 ± 5.3 minutes. This

suggests that if there is a recovery process during optogenetic suppression of auditory cortex, it lasts at least 20.5 minutes. Given the lack of any trend towards recovery (Fig. 3g), it seems likely that complete recovery would take appreciably longer than 20.5 minutes.

Given that we observed a complete recovery following electrolytic lesions of auditory cortex, tested 4 hours after lesion, these results suggest that either (1) recovery after cortical lesion/suppression has a time course that lies between 20.5 minutes and 4 hours, or else (2) electrolytic lesions and optogenetic suppression have fundamentally different effects on tone discrimination performance. To distinguish between these possibilities, we designed a sustained optogenetic protocol to mimic as closely as possible the time course of our electrolytic lesion experiments.

Sustained Optogenetic Suppression Impairs Tone Discrimination

In our electrolytic lesion experiments, we tested well-trained mice for half an hour in the morning to establish same-day baseline performance, then electrolytically lesioned auditory cortex bilaterally (45 seconds of current), and re-tested mice as soon as they were bright, alert, and responsive (median 4 hours post-lesion). To optogenetically mimic this protocol, we replaced the electrolytic lesion step with an onset of sustained 100 Hz laser illumination. We ran mice on tone discrimination for half an hour, then connected fibers and started a sustained 100 Hz laser pulse train, and allowed mice to remain in a holding cage in the testing chamber for 4 hours of sustained 100 Hz illumination.

Figure 3 (next page). Cycle suppression

a. We designed a cycle suppression protocol in which a 100 Hz laser pulse train (300 mW/mm^2, 5 ms on/off or 50% duty cycle) was on for 20 consecutive trials, and then off for 20 consecutive trials, in alternating blocks. Blocks of 20 trials had median durations of 1-3 minutes.

b. Electrophysiological validation of optogenetic suppression using a 100 Hz laser pulse train in separate mice. We used a fixed duration of 100 seconds (corresponding to the approximate duration of 20 trials) and measured the effect on spontaneous activity in silence (10 repetitions, n = 153 neurons). The suppression of spontaneous activity was significant and stable throughout the duration of the pulse train.

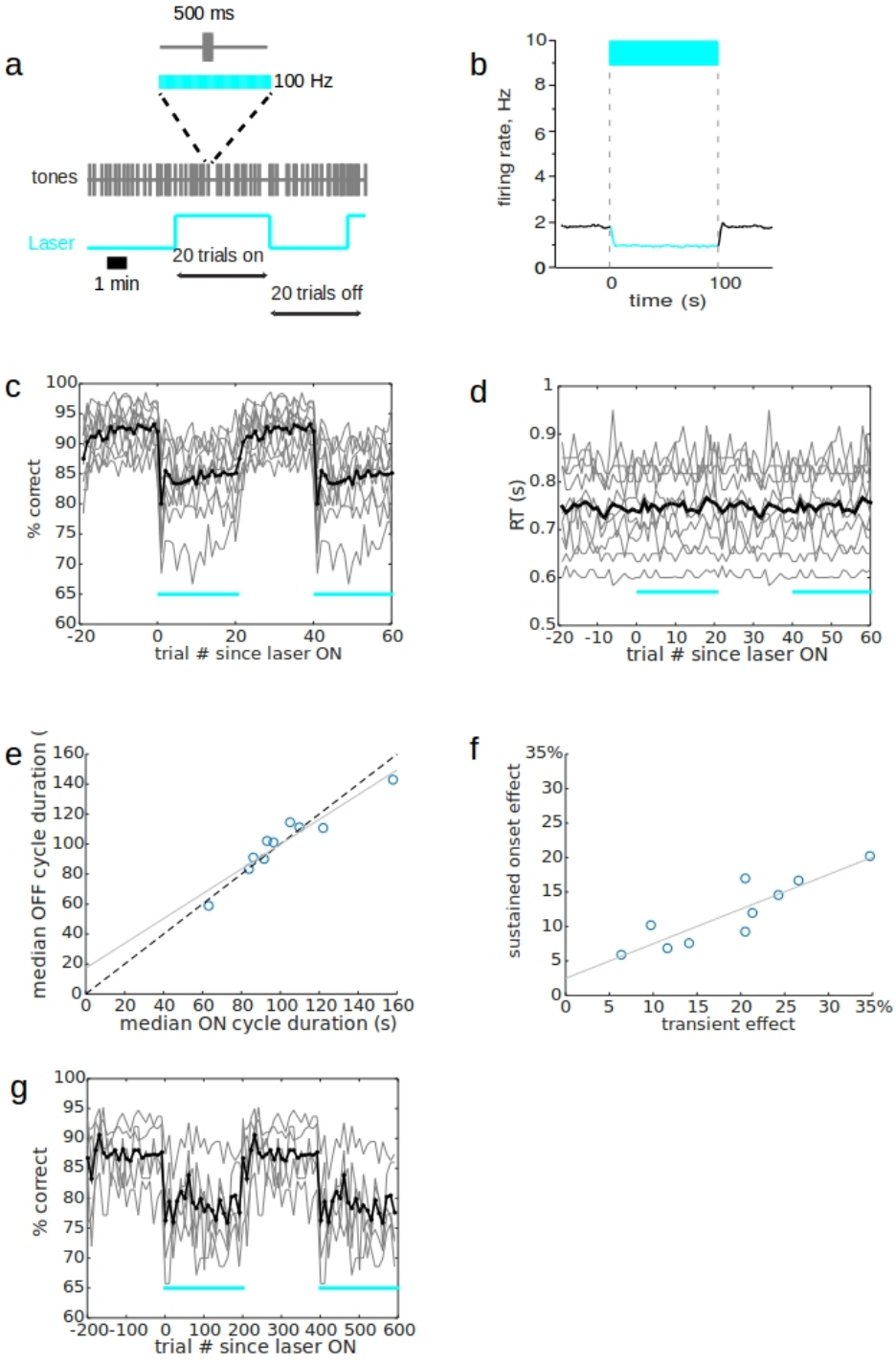
c. Performance on each trial within the cycle, averaged across a minimum of 119 blocks, is shown for individual mice in gray, with the mean across all mice in black (n = 10 mice). Cyan bars indicate the trial blocks with the laser on. The data is duplicated to display two complete cycles. Mice showed a sharp initial drop in performance on the first trial in an on-cycle, followed by a rapid but partial recovery. Performance did not return to baseline performance by the end of the 20 trial block.

d. Median reaction time on each trial within the cycle, averaged across all cycles, is shown for individual mice in gray, with the median across all mice in black. Reaction times were unaffected by laser.

e. Median 20-trial cycle duration for the on and off cycles of each mouse. Median on-cycle duration and off-cycle duration were tightly correlated and nearly equal, indicating that mice showed individual differences in trial rate but no difference between response rates during on-cycles and off-cycles. Grey line is a linear regression, dashed line is unity.

f. After testing on 20-trial cycles, we re-tested all 10 mice with transient 500 ms suppression (as in Fig. 1a). We compared the effect size for cycle suppression to the effect size for transient 500 ms suppression, for each mouse. We quantified effect size for cycle suppression as the drop in performance from the last trial in the off-cycle to the first trial in an on-cycle. Effect size was tightly correlated between the two suppression protocols, indicating that mice showed stable individual differences in the effects of suppression. The effect size for transient suppression was roughly double the effect size for cycle onset. Gray line is a linear regression.

g. To test cycle suppression over a longer time course, we extended the cycle protocol shown in A to span 200 trials with the laser pulse train on, alternating with 200 trials with laser off. Performance on each trial within the cycle is shown in same format as b, except that data is binned (in 10-trial bins). Mice did not not show a full recovery to control performance even after 200 trials (median duration 20.5 minutes).


a
500 ms
100 Hz
tones
Laser
20 trials on
1 min
20 trials off
b
firing rate, Hz
time (s)
c
% correct
trial # since laser ON
d
RT (s)
trial # since laser ON
e
median OFF cycle duration (
median ON cycle duration (s)
f
sustained onset effect
transient effect
g
% correct
trial # since laser ON

Then, with continued laser illumination, we re-tested tone discrimination for at least 30 minutes. Mice showed a sustained performance deficit after 4 hours of sustained illumination (Figure 4a). Mean pre-suppression performance was 93.4 ± 1.4%, and after 4 hours of suppression was 76.6 ± 3.4% ($p = 0.0012$, one-tailed Wilcoxon signed-rank, effect size $d = 0.785$, $n = 15$ sessions in 5 mice). This effect size is slightly larger than the observed effect of transient suppression during tone presentation ($d = 0.62$). This indicates that the tone discrimination deficit produced by sustained optogenetic suppression persists for at least 4 hours. From this we conclude that electrolytic lesions and optogenetic suppression have fundamentally different effects on tone discrimination performance: lesions of auditory cortex have no effect on performance as soon as it can be measured, but suppression over the same time course produces a lasting deficit.

Discussion

Here we used both optogenetic suppression and lesions to ask whether auditory cortex is required for frequency discrimination of pure tones in mice. We found that lesions of auditory cortex had no effect on tone discrimination, even when tested only 4 hours after the lesion. However, optogenetic suppression of auditory cortex significantly impaired tone discrimination, across a wide range of durations of suppression. Transient suppression just during tone presentation produced the strongest deficit. Performance during sustained suppression recovered rapidly after the first trial of suppression, but only partially, quickly stabilizing within a few trials to a persistent deficit.

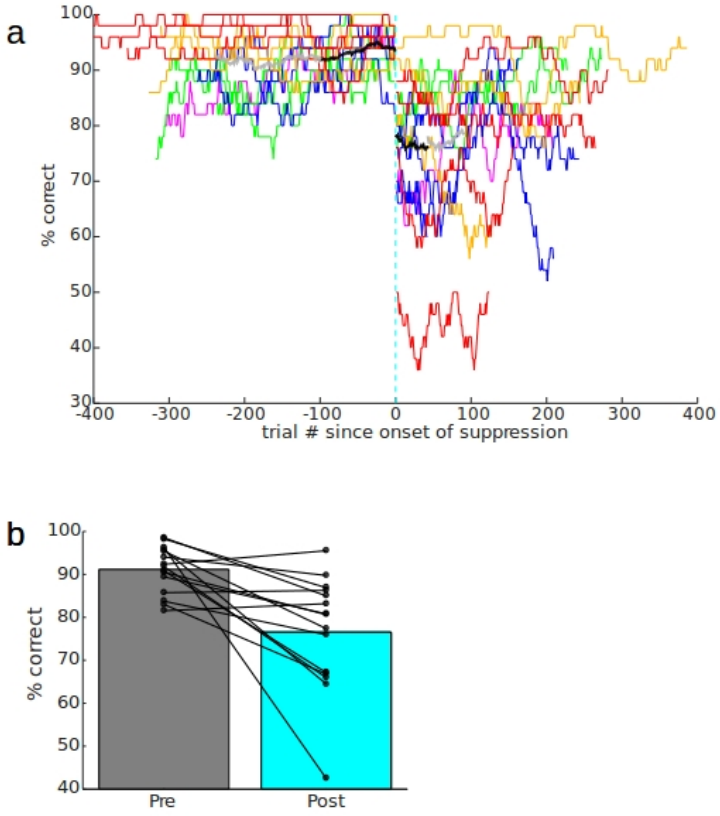


Figure 4. Sustained optogenetic suppression impairs pure tone discrimination
a. Performance for each of 5 mice before and after 4 hours of sustained optogenetic suppression (format as in Figure 2c; each mouse is shown in a different color). Note that 4 hours elapsed during sustained suppression (cyan dashed line), after which suppression was maintained throughout a second session of behavior. We repeated this experiment 1-4 times per mouse, resulting in 15 sessions for the 5 mice. Because mice ran a different number of trials in each session, we averaged across mice by including only trials contained in all 15 sessions (black line, which is limited to the duration of the shortest session). The grey line shows the average across the 14 sessions not including the shortest one.
b. Mean performance before and after sustained suppression. Format as in Figure 2d. Connected dots show each mean performance for each of 15 sessions (using trials from the black segments in a).

This deficit remained for as long as suppression was maintained, up to at least 4 hours. These results suggest that lesions and optogenetic suppression of auditory cortex produce fundamentally different effects on tone discrimination performance, which cannot be explained simply by the time course of some recovery process. Rather, it seems likely that multiple redundant systems must contribute to tone discrimination. This suggests that the absence of auditory cortex induces switching between these systems, but that the efficacy and time course of this switching process depends strongly on the method by which auditory cortex is made unavailable. We conclude that auditory cortex does contribute to frequency discrimination for pure tones. This is surprising given that we and others have shown that lesions have no effect on tone discrimination.

Numerous lesion studies over the past 75 years have investigated the role of auditory cortex in frequency discrimination (Meyer and Woolsey, 1952; Butler et al., 1957; Thompson, 1960; Goldberg and Neff, 1961; Kelly, 1970; Sellick, 1983; Buser and Imbert, 1992; Ohl et al., 1999; Talwar et al., 2001; Rybalko et al., 2006; Porter et al., 2011; Gimenez et al., 2015). Although there are conflicting results, the consensus view is that frequency discrimination is not affected by lesions of auditory cortex, even extensive lesions of all known auditory cortical fields. Cortical lesions have been shown to produce deficits if the stimuli are more complex (such as frequency-modulated tones, or complex tones with a missing fundamental frequency), or if a temporal judgement is required, or with greater task difficulty or more elaborate testing or training procedures. For pure tones, however, it appears that some other brain region or system can support frequency discrimination when auditory cortex is destroyed. In contrast, transient inactivation of rat

auditory cortex with muscimol has been reported to completely eliminate tone detection (as if animals were totally deaf), with coarse frequency discrimination recovering over several hours as muscimol gradually wears off, followed by recovery of fine frequency discrimination (Talwar et al., 2001). It has been difficult to reconcile these strikingly different effects of lesions and muscimol. One possible explanation is that muscimol, a small molecule, could diffuse to and inactivate other cortical or subcortical auditory structures. Yet auditory cortical lesions have been shown to produce extensive degradation of the MGN (Kelly, 1970), without impacting tone discrimination, suggesting that muscimol must diffuse at least to inferior colliculus or brainstem structures in order for diffusion to account for the difference from lesion results. Other studies have reported that muscimol inactivation of auditory cortex had only a slight (but significant) impact on frequency discrimination (effect size ~6%) (Gimenez et al., 2015). An important difference between these studies is that Talwar et al. used a 20-fold higher dosage of muscimol, which could support greater diffusion (but not a difference in the degree of inactivation of auditory cortex, which was nearly complete in both studies). Other differences such as task design and drug administration methods could also play a role. A different possible explanation for the discrepancy between lesion and some muscimol inactivation studies is that the recovery process, or switching of task implementation from auditory cortex to some other brain structure (such as inferior colliculus), is engaged by injury but not by inactivation. Our results support this interpretation. Our findings that optogenetic suppression impaired tone discrimination, but lesions did not, even when the time course was similar for both (4 hours), are

consistent with the idea that injury induces a rapid recovery process that inactivation alone does not. The fact that the time course, task design, behavioral apparatus, and species were identical for both our lesion and optogenetic suppression experiments greatly constrains the possible factors that could lead to recovery or the lack thereof.

Our results indicate a role for auditory cortex in tone discrimination, but it remains unclear what the nature of this role is. For example, optogenetic suppression of auditory cortex could produce a deficit in sensory processing, or could instead interfere with the integration of the sensory stimulus and the appropriate motor response (Kuchibhotla and Bathellier, 2018). For example, it is possible that both auditory cortex and an alternative circuit normally operate to discriminate frequencies, but the alternative circuit is ignored for the purposes of decision-making as long as there is some auditory cortical activity on which to base a choice. At the sensory processing level, there are conflicting reports about whether lesions of auditory cortex affect hearing thresholds (Butler et al., 1957; Goldberg and Neff, 1961; Neff et al., 1975; Heffner and Heffner, 1986, 1990). We designed our stimuli to be easily discriminable, with a sound level of 77 dB and a wide >1.5 octave frequency separation. This suggests that a change in hearing thresholds would need to be substantial in order to account for our results. The fact that suppression had no effect on response times (Figure 3d-e) helps to rule out effects on motivation or arousal, or effects due to distraction or motor disruption.

A role for auditory cortex in frequency discrimination has also been shown by modulation of cortical activity, rather than complete suppression. Interestingly, moderate photoactivation of PV interneurons in auditory cortex, using a PV-ChR2 strategy similar

to ours, has been shown to improve frequency discrimination acuity (Aizenberg et al., 2015). In that study, PV activation using a low laser power (0.2 mW/mm^2) had a stronger effect on spontaneous than on tone-evoked activity, leading to a net increase in tone-evoked signal-to-noise ratio. This suggests that enhanced population coding in auditory cortex underlies the improvement in frequency discrimination thresholds seen with low-power photomodulation (Aizenberg et al., 2015; Briguglio et al., 2018). In contrast, we used a thousand-fold greater laser power (300 mW/mm^2), which abolished tone responses altogether rather than enhancing signal-to-noise.

One mechanism that has been proposed to explain differing effects of lesions and optogenetic suppression is the loss of a biasing input to downstream structures (Otchy et al., 2015; Hong et al., 2018). In this scenario, loss of activity in auditory cortex would cause a loss of synaptic drive to downstream neurons, acutely dropping their membrane potentials below firing threshold and impairing the flow of information through circuits supporting discrimination performance. Due to homeostatic mechanisms such as an increase in membrane excitability, these downstream neurons respond to a sustained lack of firing by lowering spike thresholds, allowing a previously sub-threshold alternative synaptic pathway to drive spiking output. Homeostatic synaptic plasticity or shifts in the balance of excitation to inhibition could achieve similar results (Scholl and Wehr, 2008). Thus acute optogenetic suppression could impair circuit function, but on a longer homeostatic time scale, alternative circuits could support recovery from a lesion. Key to this model is the difference in time scale between acute optogenetic suppression and longer-term recovery from a lesion. Our results appear to rule out this scenario, because

in our sustained suppression experiment we maintained optogenetic suppression for as long as animals took to successfully recover from a lesion and perform without deficit. This indicates that any homeostatic mechanism arising from a loss of cortical activity should have had enough time to permit recovery through an alternative pathway.

Similar to our findings, optogenetic suppression of somatosensory cortex partially impairs a whisker-based object detection task (Hong et al., 2018). However, somatosensory cortical lesions also produced a partial impairment on that task, in contrast to our findings. Interestingly, object detection performance recovered abruptly to pre-lesion levels by the next session, and task exposure appeared to be instrumental for this recovery. We found that mice showed no deficit after lesions, even within the first few trials (Fig. 2b,c), suggesting that task exposure is not necessary for recovery under our experimental conditions. Dorsolateral striatum appeared to be necessary for recovery after somatosensory cortical lesions (Hong et al., 2018), raising the possibility that the striatum might also be involved in the rapid recovery of tone discrimination ability after lesions to auditory cortex.

Bridge to Chapter III

To expand on the knowledge gained in the experiments contained in Chapter II, Chapter III extends the experimental paradigm to examine the necessity of auditory cortex for discrimination of more complex sounds. Additionally, Chapter III involves the examination of long-term effects of learning related to laser stimulation.

CHAPTER III:

AUDITORY CORTEX CONTRIBUTES TO PHONEME DISCRIMINATION

JOURNAL STYLE INFORMATION: pending submission to Frontiers in Neuroscience
AUTHOR CONTRIBUTION: Conor T. O'Sullivan, Aldis Weible, Mike Wehr

This chapter is a manuscript that will be submitted to Frontiers in Neuroscience. In this paper, I was the primary person responsible for experimental design, data collection/analysis, and the bulk of writing. I received assistance from Aldis Weible, who carried out and analyzed electrophysiology experiments and surgical procedures. Mike Wehr, the final author, is my lab's PI and assisted throughout the experiment and writing process.

Introduction

Speech activates broad spatiotemporal patterns of activity throughout the mammalian auditory system (Johnson et al., 2005; Kilgard and Engineer, 2015; Liebenthal et al., 2005). The distinct neural representations evoked by different speech sounds is thought to underlie our ability to distinguish between them (Centanni et al., 2013; Engineer et al., 2008; Kilgard and Engineer, 2015). However, it is still unclear which details of these spatiotemporal activity patterns matter, and how neural processing of them leads ultimately to perceptual discrimination. Early in the auditory pathway, speech-evoked neural activity appears to encode the acoustic structure of speech sounds, whereas in higher order auditory areas it appears to encode progressively more

categorical aspects of speech (Chang et al., 2010; Flinker et al., 2011; Pasley et al., 2012; Tsunada et al., 2011).

It is clear from lesion studies that auditory cortex is necessary for the discrimination of complex sounds, including speech, but not for simpler auditory tasks such as frequency discrimination (Cranford et al., 1976; Floody et al., 2010; Ohl et al., 1999; Porter et al., 2011). Converging evidence from electrophysiology, behavior, and lesion studies has implicated precise spike timing during the initial onset response in auditory cortex as being especially important for the discrimination of speech sounds. In rats trained to discriminate consonants, their discrimination performance is well-correlated with the distinctness of spatiotemporal activity patterns in auditory cortex (Centanni et al., 2013; Engineer et al., 2008). Precise spike timing is critical for this relationship, because the removal of spike timing information by binning responses disrupts both neural discriminability and its correlation with behavioral discrimination (Centanni et al., 2013; Engineer et al., 2008)(Schnupp et al., 2006). The initial 40 ms of the onset response in auditory cortex appears to be particularly informative. For neural decoding of speech sounds, the initial 40 ms of the response contains the most information required for neural discrimination of consonants (Centanni et al., 2014; Engineer et al., 2008; Perez et al., 2013). In addition, auditory cortex lesions cause much greater impairment of the discrimination of speech sounds that are truncated to the initial 40 ms than for full syllables (Floody et al., 2010; Porter et al., 2011).

The picture that emerges from these findings is that not all of the neuronal spiking in a speech-evoked spatiotemporal pattern of activity is equipotent for discrimination of

consonants. Rather, early activity appears to contain more discriminative information than later activity. However, the hypothesis that early cortical activity matters more for discrimination of speech sounds than late activity has not yet been rigorously tested. Speech-evoked responses of cortical neurons show higher correlation with behavioral discriminability during the initial 40 ms of activity (Engineer et al., 2008), but this correlation does not demonstrate a causal relationship with behavioral discrimination. The fact that cortical lesions cause greater impairment for discrimination of truncated speech sounds demonstrates a causal role for auditory cortex (Porter et al., 2011), but manipulating the stimulus is not the same thing as manipulating the neural representation itself. Here we set out to test this hypothesis by taking advantage of the temporal precision of optogenetics to manipulate cortical activity during the discrimination of speech sounds. Mice discriminated the words "dad" and "sad," pitch-shifted up into the mouse hearing range. These sounds are most different in the early 0-140 ms time window (initial consonant "d" or "s") but are much more similar in the late 140-280 ms time window (during the rhyming vowel sound). We predicted that suppressing early speech-evoked activity in auditory cortex would cause a greater impairment than suppressing late activity. We found that full suppression over the entire duration of the stimulus significantly impaired discrimination, confirming previous results from lesion studies. Surprisingly, we found that suppression during the early or late temporal windows were equally disruptive, each causing as much impairment as full suppression. We conclude that for mice trained to discriminate speech sounds, disruption of any temporal component of the representation impairs discrimination. Our interpretation is that

regardless of which spatiotemporal aspects of a representation may be most informative, any type of disrupted representation differs from those learned in training, thereby impairing performance.

Methods

All procedures were performed in accordance with the National Institutes of Health guidelines, as approved by the University of Oregon Institutional Animal Care and Use Committee.

Mice

In this study, we used a total of 9 mice for optogenetic suppression of auditory cortex during performance of a phoneme discrimination task. The mice were offspring from a cross of homozygous Pvalb-IRES-Cre ("PV", JAX No. 008069; The Jackson Laboratory) and homozygous CAG-ChR2-eYFP ("ChR2", JAX No. 012569; The Jackson Laboratory) lines, which are on a C57Bl6/J background. In these mice ("PV-ChR2"), ChR2 was expressed in parvalbumin-expressing (PV+) interneurons (Moore 2013). We used an additional 2 PV-ChR2 mice (not used for behavior) for electrophysiological validation of optogenetic suppression.

Surgical procedures

To deliver laser illumination to auditory cortex while mice performed the task, we surgically implanted optical fibers bilaterally before the beginning of training. We

administered dexamethasone (0.1 mg/kg) and atropine (0.03 mg/kg) pre-surgically to reduce inflammation and respiratory irregularities. Surgical anesthesia was maintained with isoflurane (1.25-2.0%). We implanted 200 μm optic fibers in each hemisphere at AP ~2.3 mm (relative to bregma), ML 4.4 mm, and depth 0.5 mm below the dura (just dorsal to primary auditory cortex). The implants were painted with black acrylic paint to minimize light leakage. For electrophysiological verification of optogenetic suppression, we implanted 2 mice (not used in behavioral experiments) with a unilateral optrode array, consisting of 8 tetrodes and a 200 μm fiber terminating 1 mm above the recording sites. The 8 tetrodes passed through two 28-gauge stainless steel hypodermic tubes, with 4 tetrodes per tube. The optic fiber was fixed in position immediately adjacent to, and between, these tubes. Tetrodes were made of 18 μm (25 μm coated) tungsten wire (California Fine Wire). The entire array was mounted on a custom microdrive. The optrode array was inserted vertically through a small craniotomy (2 mm x 1 mm) dorsal to auditory cortex, and cemented into place. We administered ketoprofen (4.0 mg/kg) post-operatively to minimize discomfort. Mice were housed individually following the surgery and were allowed 7 days of post-operative recovery.

Histology

Brains of mice used for electrophysiological validation were sectioned (100 μm) in the coronal plane to verify the position of single neuron recording sites. Only data corresponding to tracks located within auditory cortex were included. Following behavior

experiments, 6 out of the 9 mice were sectioned to confirm the location of implanted fibers in auditory cortex.

Behavioral apparatus

Mice performed the task in sound-attenuating behavioral chambers. Within the chamber, mice were placed in a plastic arena, one wall of which contained 3 combination ports for lick-sensing and water delivery (Fig. 1a). Each port had an IR beam-break sensor, at which mice responded by licking, and a tube to deliver water rewards for correct responses. Sound stimuli were controlled by a computer running custom behavioral software (modified from Meier et al., 2011), and delivered through 2 speakers placed outside the arena facing the ports. Since laser illumination was delivered with blue light that could potentially be visible to the mouse, we used a color-matched blue strobe light (full-field illumination at approximately 10 Hz) to mask laser stimulation. Mice were trained for an hour each day for 5-7 days a week, corresponding to 300-500 trials and 1-2 g of water reward per day. Mice were water-restricted, typically receiving water only through performance of the task, but were supplemented as necessary to remain above 80% of pre-training body mass.

Stimuli

We recorded the words “sad” and “dad”, spoken by a female native speaker of US English, using a Bruel & Kjaer 4939 microphone. We digitized the signal at 176 kHz and then decimated down to 44.1 kHz. Because much of the frequency content of human

speech is below the mouse hearing range (which is approximately 1-80 kHz), we pitch-shifted the speech upwards by one octave using a frame-based algorithm with fourier transforms of window length 2048, analysis length 512, and synthesis length 1024 samples (Saunders and Wehr, 2019). The resulting pitch-shifted stimuli had preserved temporal structure and a sample rate of 88.2 kHz (Fig. 1b). Because the initial consonants /s/ and /d/ were of different durations, we added 50 ms of silence to the beginning of the "dad" stimulus so that the consonant-to-vowel transition occurred at approximately 140 ms for both stimuli, and both stimuli had a total duration of 500 ms.

Task structure

Table 2: Training steps

Step	Description	Advancement
1. Surgery	Fiber implantation	1 day of water restriction post-recovery
2. Free drinks	Ports give water, no stimulus	Trial rate
3. Request Rewards	Rewards for center port trial requests & correct responses	Trial rate
4. Only correct rewards	Request rewards disabled	400 trials completed
5. Long penalty	Increased timeout for incorrect responses	Performance > ~70%
6. Optogenetic suppression	Laser on for 10% of trials	N/A

Prior to any behavioral training, the mice underwent surgical implantation of optical fibers (Step 1, see Table 2). After recovery, the mice were familiarized with the operation of the ports in the absence of sound stimuli using a simple lick-for-water task (Step 2, “Free drinks”). Next, they advanced to the first stage of the main task (Step 3). In the main task, mice requested trials by licking the center port, which triggered stimulus delivery. Mice responded by licking at the left port (for “sad”) or the right port (for “dad”). Correct responses triggered an 80 µl water reward followed by a 1 second delay before the next trial could be requested, whereas incorrect responses gave no water and provided an additional 1 second penalty timeout before the next trial. To increase the number of trials performed, some mice had their water rewards reduced to 60 or 40 µl. During an initial shaping stage of the main task (Step 3), mice received water rewards at the center port for requesting trials (as well as for correct answers at the side ports) until reaching a rate of 7 completed trials in 30 seconds. Once the mice achieved this rate of trials, the rewards for center-poke trial requests were removed, forming the next stage (Step 4) of the task structure. After 400 trials in this condition, the penalty timeout for incorrect responses was increased to 3 s (Step 5). In Steps 3-5 we included “correction trials” to reduce the development of response bias to one side or the other. After an incorrect response, there was a 50% chance that a mouse would go into a correction trial sequence, in which the same stimulus was repeated until the mouse responded correctly. Correction trials provide contextual information that could conceivably allow a task strategy that did not depend solely on stimulus discrimination, so we disabled correction trials during the final optogenetic suppression stage (Step 6). When mice were

performing at 70% or higher on Step 5 for approximately 5 days, they were run for at least 2 days with fibers attached but without light delivery, to allow the mice to become accustomed to the fibers. Then mice advanced to the final stage (Step 6) for optogenetic suppression experiments.

Optogenetic Suppression

To suppress auditory cortex, we delivered 445 nm wavelength laser pulses to auditory cortex through chronically implanted bilateral optical fibers. We used two laser powers: a standard total power of 20 mW (corresponding to 630 mW/mm^2 at the fiber tip), and additional testing with a total power of 9.5 mW (300 mW/mm^2). In a previous study using identical fiber implantation and lasers, we electrophysiologically characterized the spatial extent of cortical suppression, which was 1750 μm at a power of 9.5 mW (Weible et al., 2014). We have not characterized the spatial extent of suppression at 20 mW, but based on our previous measurements at 9.5 mW and a model of light transmission in mammalian brain tissue (DeisserothLab), we estimate that the spatial extent of suppression at 20 mW is 2100 μm, an extent that includes all tonotopic fields of auditory cortex, throughout the cortical depth, but does not include thalamic, collicular, or other subcortical auditory structures. Laser illumination trials were randomly interleaved on 10% of trials. We used three temporal profiles for illumination. For “Full” suppression trials, illumination was delivered during the entire 500 ms from sound onset to sound termination. For “Early” suppression trials, illumination was delivered from 0-140 ms following stimulus onset, whereas for “Late” trials, illumination was delivered

from 140-280 ms following stimulus onset (Fig. 2a). Because the consonant-to-vowel transition occurred at ~140 ms, Early suppression coincided with the initial consonants (/d/ or /s/), and Late suppression coincided with the following vowel. We did not include a condition that specifically targeted the trailing consonant /d/. Initially, all laser trials were rewarded randomly in order to avoid the possibility that mice might be able to learn new associations between laser+stimulus and rewards (if laser+stimulus combinations were correctly rewarded according to the stimulus identity). In follow-up experiments, we explicitly examined whether mice could learn new associations by correctly rewarding laser trials.

As an additional control experiment, we tested the possibility that visible cues produced by the laser could influence performance through learning. In this case, instead of connecting the optical fibers to the implanted optical ferrules, we placed them on the wall of the behavioral chamber, where the light was clearly visible to the mouse, but not reaching the brain. We tested this condition with both randomly rewarded laser trials and correctly rewarded laser trials to examine the ability of mice to learn visible laser-cue associations under both reward conditions.

Statistical Analysis

We calculated performance separately for responses on laser and control trials. Because not all performance data were normally distributed (Lilliefors test), we used non-parametric statistics throughout. We compared accuracy (in percent correct) for individual mice using Fisher's exact test on the contingency tables created by the two

stimuli and two possible responses, using the odds ratio as a measure of effect size. We tested for group effects using the two-tailed Wilcoxon signed-rank test. Because we found a range of long-term learning effects (as described below in Results, "Laser-related learning" and in Fig. 3), we used only the first 10,000 total trials after the start of laser stimulation (Step 6) unless otherwise noted. To examine changes over time, we calculated performance using a 200-trial sliding window.

To determine the rightward or leftward response bias of each mouse, we calculated the difference between the proportion of rightward responses and rightward stimuli (i.e., stimuli for which the correct response is rightward) in a sliding 200-trial window. A difference of zero indicates no bias, such that responses are proportionate to the stimuli presented. For display, we added 50% to the bias values, such that 50% indicates no bias, values <50% indicate leftward bias, and values >50% indicate rightward bias.

For receiver-operator-characteristic (ROC) analysis (Green and Swets, 1966), we evaluated performance separately for each stimulus and then compared between laser and control trials. To show stimulus-related effects, each mouse's performance was separated into "hit rate" (percent correct on "dad" trials) and "false alarm rate" (100 - percent correct on "sad" trials). This arbitrary assignment to hits and false alarms allows ROC analysis of laser effects on both accuracy and response bias. For each mouse, we plotted hits against false alarms separately for laser and control conditions. The distance between these points indicates the magnitude of the laser effect on accuracy, and the direction between them indicates the degree of induced bias.

Single Neuron Recording and Analysis

Tetrode data were acquired with 32-channel RHD2000 hardware (Intan Technologies) and Open Ephys software (Siegle et al., 2017). A minimum threshold of 60 μV was set for collection of spiking activity. Activity of individual neurons was isolated offline using MClust (Redish, 2008). Measures of peak and trough waveform voltage, energy, and principal components analysis were used as waveform separation parameters in 2-dimensional cluster space. Cells were accepted for analysis only if they had a cluster boundary completely separate from adjacent cluster boundaries, and completely above threshold, on at least one 2-D view. Cluster boundaries were then applied across sessions to track single cell responses across different stimulus contingencies.

We recorded neuronal responses to the pitch-shifted "sad" and "dad" stimuli used for behavior (500 ms duration, 500 ms inter-trial interval, 50 repetitions) with or without optogenetic suppression on interleaved trials. We used the same laser power as in the behavior experiments (20 mW and 9.5 mW, corresponding to an irradiance of 630 and 300 mW/mm^2 as measured at the tip of the 200 μm diameter fiber). All data were collected as mice freely explored a cylindrical plastic container (height 16 cm, diameter 16 cm) inside a double-walled sound-attenuating chamber. Sounds were delivered from a free-field speaker directly above the cylinder. The speaker was calibrated to within ±1 dB using a Brüel and Kjær 4939 1/4-inch microphone positioned within the cylinder approximately at head height. Following each recording session, the tetrode array was lowered ~80 μm and allowed to settle for a minimum of 3 hours before initiating another session to ensure that responses collected during each session reflected the activity of a

unique population of cells. Recordings from putative PV cells, as identified by significant firing rate increases during laser pulses in silence compared with an equivalent period of silence with laser off (paired t-test), were excluded from group analyses.

Results

Training

Out of an initial 15 mice cohort implanted and trained on the task, 9 successfully learned the task, reaching and maintaining 70-80% performance. From the start of training, it typically took 25-30k trials before mice advanced to optogenetic suppression experiments, corresponding to approximately 2-3 months.

Electrophysiology

To verify that our optogenetic method effectively suppressed cortical activity, we recorded from auditory cortical neurons in 3 separate mice (not used for behavior) using a tetrode array, attached to an optical fiber implanted in the same location as the mice used for behavior. We recorded from 116 neurons. We excluded 9 PV cells, which were unambiguously identified by robust responses to illumination (Moore and Wehr, 2013). Figure 1c shows a typical example of responses to the “dad” and “sad” speech stimuli, with complete optogenetic suppression by 300 mW/mm^2 (9.5 mW) laser illumination. Across the population, suppression was nearly complete for both 300 and 630 mW/mm^2 laser illumination (Fig. 1c). Because neurons varied in the timing of their responses to the speech stimuli, the brisk transient responses seen in individual cells (Fig. 1c, top) are not

as evident in the population-averaged response (Fig. 1c, bottom). However, the population average shows the effectiveness of optogenetic suppression. Across the population of 107 non-PV cells, suppression reduced the mean firing rate during phoneme presentation from 5.8 ± 5.5 to 0.6 ± 1.6 Hz (mean ± s.d., $t = 18.8$, $p < 0.0001$, paired t-test) at an intensity of 300 mW/mm^2, and from 5.1 ± 5.4 to 0.4 ± 1.1 Hz (mean ± s.d., $t = 17.4$, $p < 0.0001$) at an intensity of 630 mW/mm^2. Responses were significantly suppressed by illumination in 100/107 (93%) non-PV neurons.

Full Stimulus Suppression

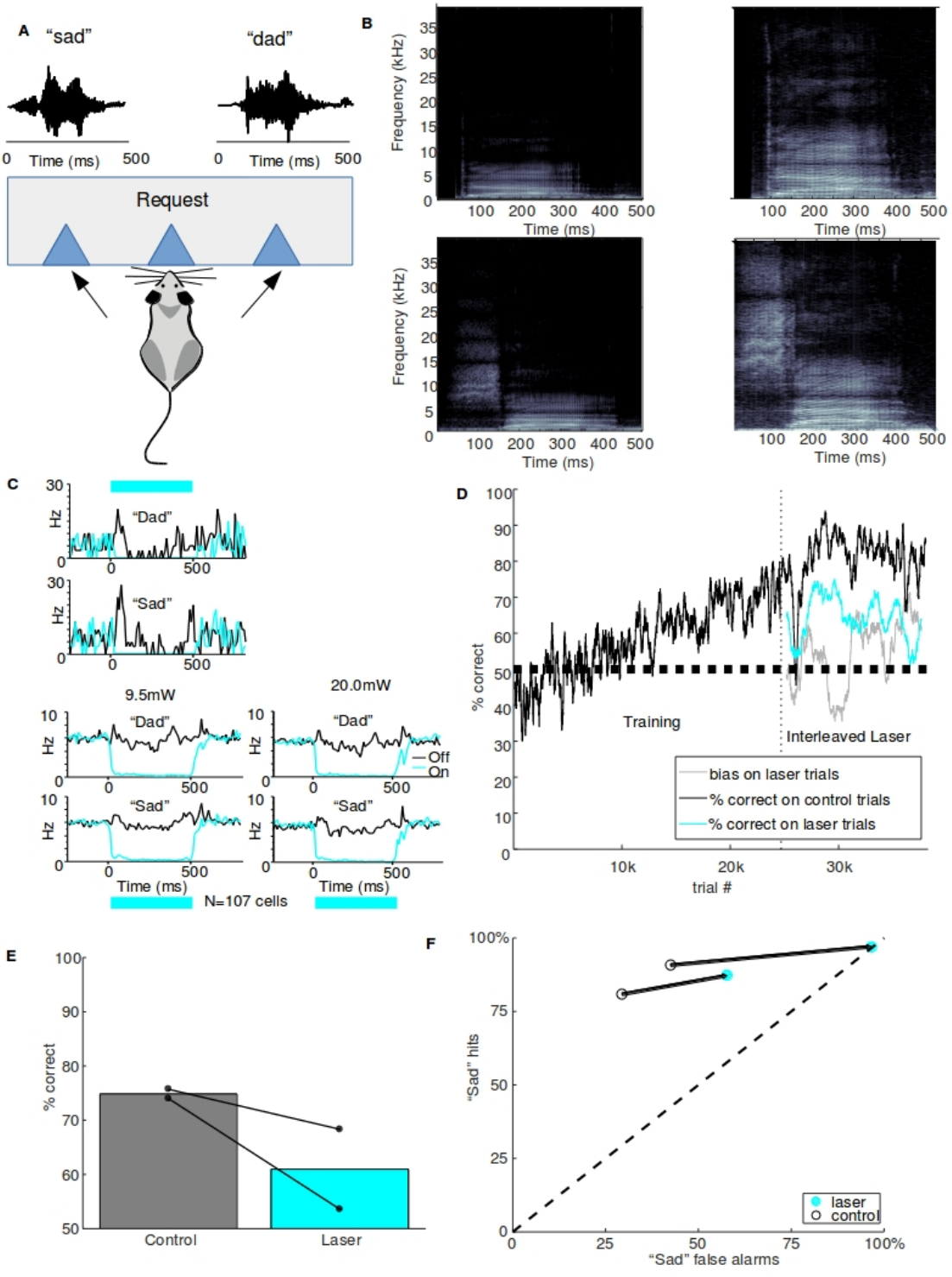
We first tested two mice, with an example training course shown in Fig. 1d, with "Full" stimulus laser suppression, in which 20 mW illumination was delivered during the entire 500 ms from sound onset to sound termination. Laser trials were randomly interleaved on 10% of trials. Performance was significantly impaired on laser trials compared to control trials (Fig. 1e, 74.8 ± 2% on control trials, 60 ± 7% on laser trials. Both mice showed significant individual effects using the Fisher's exact test (mouse 5623: $p<0.0001$, mouse 5625 $p =0.0002$). Since there was a significant deficit when auditory cortex was suppressed, these results show the necessity of auditory cortex activity for phoneme discrimination.

Multiple Suppression Windows

We used two speech stimuli that rhyme ("dad" and "sad"). That is, the initial consonant differs (/d/ vs. /s/), but the vowel and final consonant are the same ("ad"). We

therefore wondered whether neural activity evoked by the initial consonant might be more informative for discrimination than neural activity evoked by the vowel and final consonant. Even though the acoustic fine structure of the "ad" is slightly different in the two tokens, we reasoned that they are much more similar than the initial consonants, and therefore that the neural activity they evoke might provide less discriminative information.

Figure 1 (next page): Transient optogenetic suppression of auditory cortex during the full stimulus duration impaired phoneme discrimination.
a: The port mapping for this task involved a central request, with the waveforms for the "sad" and "dad" stimuli shown above their associated response port.
b: Spectrograms of the original human voice recordings of "dad" and "sad" are shown alongside the pitch-shifted (up one octave) phonemes used for behavioral testing. In all plots, grayscale represents the power spectrum density (dB/Hz), with black representing -110 and white representing -30.
c: Tetrode recordings were used to observe normal phoneme responses and their suppression with 9.5 and 20mW laser power. The 500ms stimulus and laser suppression begin at the 0ms point. The upper panel contains example data from a cell, with data averaged across both mice and all cells in the lower two panels.
d: Performance of a typical mouse from initial training stages through laser suppression. Vertical dashed line indicates the onset of laser trials. Performance is represented as percent correct averaged across both stimuli in a 200-trial sliding window. Black: performance on control trials, cyan: performance on laser trials, grey: bias on laser trials.
e: Performance in overall percent correct for each mouse is represented by connected dots representing control trials and suppression trials. Bars represent mean performance for each trial type across all mice.
f: To show stimulus-related effects, each mouse's performance was separated into "hit rate" (Percent correct on "dad" trials) and "false alarm rate" (100 - Percent correct on "sad" trials). The dashed line represents chance performance (50% correct), ranging from 100% left/sad responses at (0,0) to 100% right/dad at (100,100).


A
"sad"
"dad"
0
Time (ms)
500
Request
B
Frequency (kHz)
Time (ms)
C
"Dad"
"Sad"
Hz
9.5mW
20.0mW
Off
On
N=107 cells
D
% correct
Training
Interleaved Laser
bias on laser trials
% correct on control trials
% correct on laser trials
trial #
E
Control
Laser
F
"Sad" hits
"Sad" false alarms
laser
control

To test this possibility, we took advantage of the temporal precision of optogenetics to suppress auditory cortical activity just during the initial 0-140 ms of the stimulus (containing /d/ or /s/, which we refer to as "Early" suppression), or instead during the trailing 140-280 ms of the stimulus (containing "ad", which we refer to as "Late" suppression). Randomly interleaved Early and Late suppression trials each made up 10% of the total, with Control trials (no illumination) as the remaining 80%. We predicted that Early suppression would have a stronger effect on behavioral performance than Late suppression. We tested a group of 9 mice, including the 2 used in full suppression experiments, and instead found that both Early and Late suppression produced similar deficits in performance (Fig. 2b; Early: 60.2% ± 7.9%, Late: 59.6% ± 6.9%, Control: 72.3% ± 4.6%, n = 9 mice). Both Early and Late suppression were significantly different from Control (Early vs. Control: $p=0.0117$, Late vs. Control: $p=0.0117$, Wilcoxon signed-rank, corrected for multiple comparisons), whereas Early and Late suppression were not different from each other ($p=0.82$). We conclude that either epoch of suppression was sufficient to disrupt performance.

To examine these effects in more detail, we quantified the effects of both Early and Late suppression using ROC analysis (Fig. 2c), which allowed us to partition the effects on performance into effects on accuracy and bias. Out of the 9 mice, 7 showed significant effects of the laser in both conditions, one mouse had a significant effect only for Late suppression, and one did not show significant effects in either condition (Fisher's exact test, $p<0.05$). Most mice exhibited a side bias on laser trials, indicated by shifts in performance towards the upper-right or lower-left corners in Figure 2c. Other mice

showed almost no bias, with performance shifted towards the lower-right corner, corresponding to similar deficits for both stimuli. Comparison of the effects of Early and Late suppression for each mouse showed that the directions and magnitudes were largely similar for both suppression windows. We conclude that the effects of the two suppression windows on performance were nearly equivalent in terms of both accuracy and bias.

Laser-related Learning

Disconnected Fibers

Because laser illumination of the brain is potentially visible to the mice during

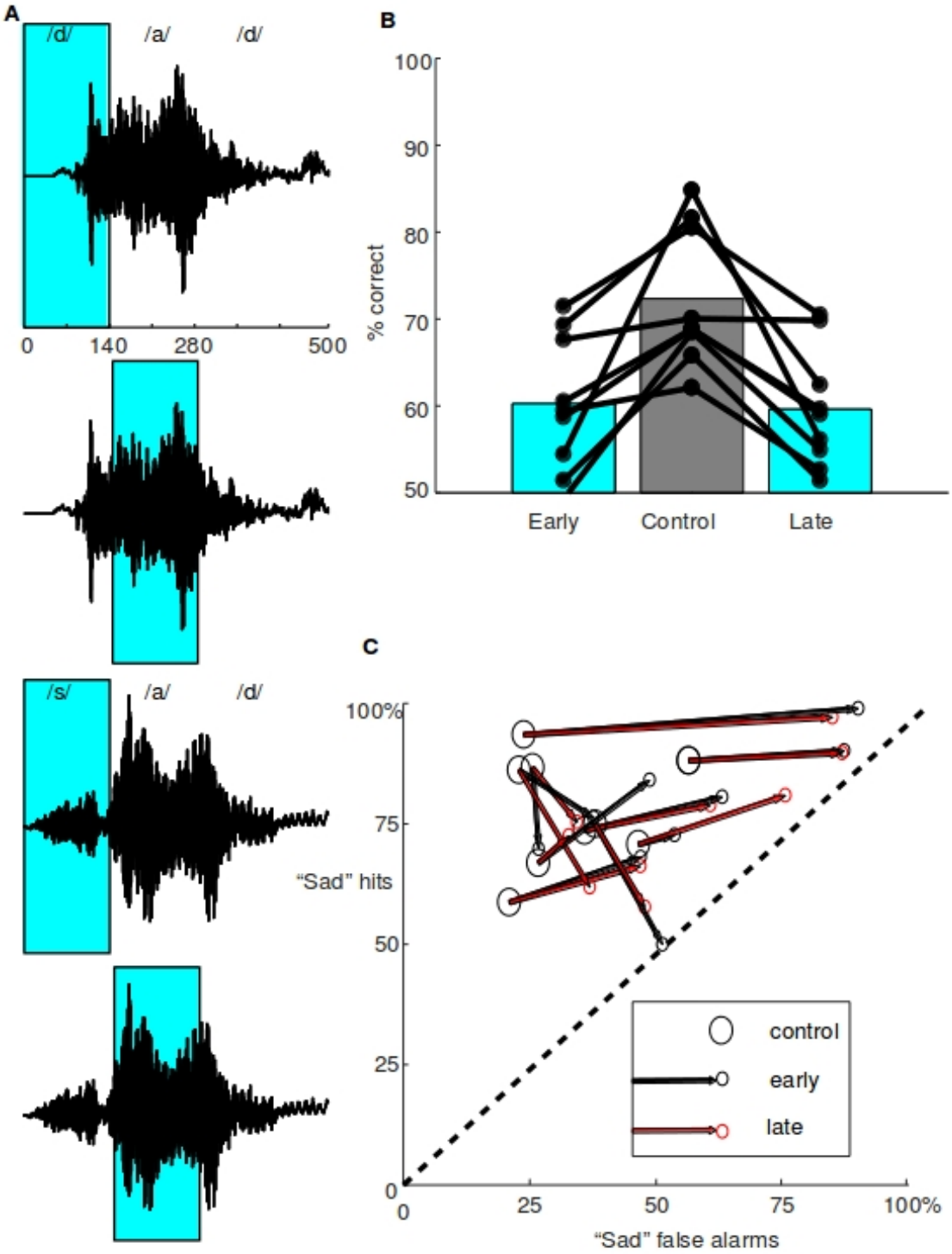
---

Figure 2 (next page): Suppression during either early or late timing windows had equivalent effects

a: The spectrograms for both stimuli ("sad" is the upper 2 panels, "dad" is the lower 2) are shown with the "early" and "late" laser suppression windows overlaid in cyan. Approximate borders for the different components of each stimulus are indicated in the top panel.

b: Performance in overall percent correct for each mouse for 10,000 total trials is represented by connected dots representing each of the 3 types of trials. Bars represent mean performance for each trial type across all 9 mice. The two laser suppression conditions, indicated by cyan bars, were timed to match either the "consonant" (early) or "vowel" (late) segment of the phoneme. Mice show similar effects of the laser regardless of its timing, shown by the general symmetry between early and late.

c: Similar to Figure 1F, performance for each mouse was broken into "hit rate" (percent correct on "dad" trials) and "false alarm rate" (100 - percent correct on "sad" trials). Each mouse is represented with a large open circle corresponding to control trials, connected to early (black) and late (red) suppression conditions. If the effect of the laser is exactly the same between the two suppression windows, the two small circles would overlap.


A
/d/
/a/
/d/
0
140
280
500
/s/
/a/
/d/
B
100
90
80
70
60
50
% correct
Early
Control
Late
C
100%
75
50
25
0
"Sad" hits
0
25
50
75
100%
"Sad" false alarms
control
early
late

task performance, we performed several control experiments on previously tested mice to distinguish between potential effects of illumination as a visual cue and direct optogenetic effects on the brain, though we did not have large enough sample sizes to characterize or quantify these effects in detail. Because we randomly rewarded laser trials, mice could potentially learn that the laser provides a visual cue that their response is irrelevant. To address this possibility, we tested mice with the laser fiber disconnected from the implanted ferrules and instead directed at the walls of the box, with illumination randomly interleaved on 20% of trials. The resulting patterns of behavior support the idea that the initial effects of brain illumination were due to optogenetic suppression of auditory cortex, but also that mice can gradually learn to associate the visual laser cue with task contingencies. For example, mouse 5982 (Fig. 3a) showed almost no difference in performance between laser and control trials for approximately the first 3000 trials during this "fiber disconnected" control condition, in which there is no direct optogenetic effect of illumination. Over time, this mouse developed a strong side bias specifically on laser trials while maintaining performance on control trials (red arrows in Fig. 3a). This is consistent with the idea that after about 3000 trials, the mouse learned that the laser cue indicated a random reward. A comparison of performance on laser trials between the first 3000 trials (75.0% correct) and the following 9000 trials (68.7%) shows a significant difference using a two-tailed Fisher's exact test ($p=0.0027$, effect size = 1.37). Mouse 5980, which had already developing a bias during the initial laser condition, was also tested in the disconnected condition (data not shown). This mouse showed a transient reversal in side bias when the laser was disconnected, but the bias then strengthened and

stabilized over time, again suggesting that the mouse learned the association of visual cue and randomly rewarded trials.

Normal Reward

We randomly rewarded laser trials to avoid the possibility that mice could learn a new association between "laser + sound" stimuli and reward. To directly address whether mice could learn new associations during suppression, and confirm the necessity of randomly rewarding laser trials, we tested the impact of switching to normal rewards on laser trials, which made up 20% of the total. Fibers remained connected to the implanted ferrules for this experiment. When mice were switched from random to normal rewards, their performance on laser trials gradually improved and eventually approached or matched control trials. Importantly, this process took at least 10,000 trials. For example, mouse 5916 gradually improved performance on both control and laser trials following the reward switch, asymptotically converging at 80% correct on both types after ~15,000 trials. 3 other mice showed less conclusive effects, but trended toward better performance or less bias following the switch to normally rewarded laser trials.

This gradual improvement in performance following a switch to normal rewards occurred whether or not the fiber was connected to the implanted ferrule. Indeed, switching to normal rewards could reverse the bias that had been learned during the disconnected but randomly rewarded condition. For example, mouse 5982 (mentioned above, Fig. 3a) developed a strong bias on randomly-rewarded laser trials with the fiber disconnected. When this mouse was switched to normal rewards (with fibers still

disconnected), this bias remained strong (~90%) for 12,000 trials but then gradually diminished as the mouse learned to respond normally on laser trials. Comparison of the proportion of rightward responses between the first 10,000 (88.6%) and remaining 32,000 (64.4%) trials showed a significant difference using a two-tailed Fisher's exact test test ($p<0.0001$, effect size = 4.31). We conclude that providing normal rewards on optogenetic suppression trials can provide reinforcement that allows some mice to learn how to correctly respond, perhaps by forming a new "laser + sound" stimulus-reward association. This can occur with visual laser cues independently of optogenetic suppression of the brain. These results suggest that in experiments testing the effects of optogenetic manipulations on operant behavioral tasks, it is important to consider the reward contingency on illumination trials. Nevertheless, because the learning of these new contingencies took at least 10,000 trials, the effects of optogenetic manipulations can be measured before new associations are learned, provided one is cautious about the potential for such learning.

Discussion

Full Stimulus Suppression

The consistent effect of reduced performance during full stimulus suppression shows the necessity of auditory cortex for phoneme discrimination. Without proper activity in AC during the stimulus, the mouse is unable to determine which phoneme it is hearing, which leads to several types of responses. Guessing randomly between the two sides and

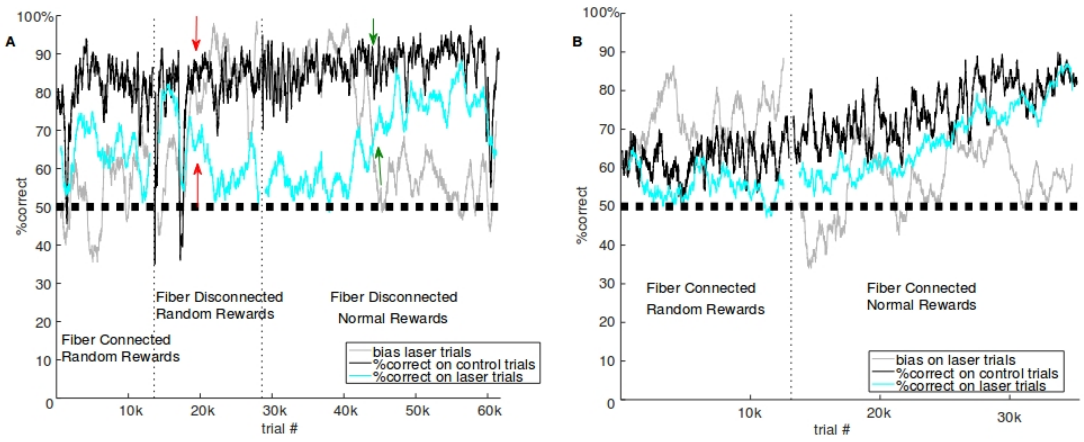


Figure 3: Mice show sensitivity to task parameters and can exhibit laser-related learning

a: Mouse 5982 went through three different forms of the task: first with standard laser suppression, then with the fiber disconnected, then finally with fiber disconnected and normally rewarded laser trials. Performance is represented as overall percent correct averaged over (100) trials, using a sliding window to cover the entire course of behavior. Performance on control trials (black) remains consistent over time, but performance (blue) and bias (gray) on laser trials are sensitive to the specific task conditions. In standard suppression, there is a ~15% drop in performance on laser trials, but when the fiber is disconnected from the brain, there is an initial period of no effect of the laser. In this condition, the visual stimulus of the laser indicates random rewards and is a salient cue, leading to a bias strategy on laser trials that minimizes effort. This developed after several thousand trials, indicated by the red arrow. In the final segment, laser trials were rewarded normally, encouraging correct performance. Over time, laser performance became indistinguishable from control as the bias diminished, with green arrows indicating the approximate time of the major shift in performance.

b: After initial testing showed a moderate effect of the standard laser suppression, mouse 5916 was tested with normally rewarded laser trials while the AC was still being suppressed. While the difference in performance during suppression was still present at the beginning of the new task structure, it was reduced over time, eventually leading to indistinguishable performance after 15k trials, showing that the mouse has learned to do the task even with AC suppression.

developing a strong bias on laser trials both provide an equal expectation of reward, since laser trials are rewarded randomly. One potential problem is the visual cue to the mouse provided by the laser, which was reduced, but not necessarily eliminated by the use of a masking strobe light. If the mouse associated the visual stimulus with a random reward, it could show reduced performance on laser trials from an effect having nothing to do with auditory cortex. We gained evidence through the unattached and normally rewarded control experiments that the initial effect was in fact related to optogenetic effects in the brain.

Multiple Suppression Windows

When activity was suppressed separately for the early (consonant) and late (vowel) segments of the phoneme, the effect of the laser remained and was equal for both epochs, suggesting that the two manipulations have a similar effect on the brain. Initially, we hypothesized that since the consonants were different (d, s) while the vowels were the same (ad) for the two phonemes, suppression during the early segment would have a stronger effect. Additionally, prior work suggested the initial 40ms provided sufficient information for discrmiination (Engineer et al., 2008). There are several reasons why there was no difference, starting with the fact that since recorded human speech is the stimulus, the exact structure of the vowel segments is not identical. In the current experiment, both segments of the phoneme could provide information used for discrimination. Future work could use synthetic stimuli, using the exact same vowel segments for both stimuli, following separately recorded consonants. However, this

would move farther from the natural structure of speech, since there is typically an effect on the structure of a vowel based on its preceding consonant.

Another interpretation is that even if the mouse was using the consonant segment, the information about the sound used to make a decision could still be in auditory cortex during the vowel segment. If this was the case, suppressing activity during both periods would impair performance, since the sound information would never reach downstream areas, such as the striatum or motor cortex, that could initiate responses.

Laser-related Learning

There are tradeoffs between the possibilities of randomly or normally rewarding laser trials, which we attempted to address. With randomly rewarded laser trials, a mouse could learn to associate the cue of the laser with its response being irrelevant, leading to spurious drops in performance. The alternative presents a different learning concern, where reinforcement on laser trials could allow the mouse to form new stimulus-reward associations. Even if the "sad" stimulus combined with suppression is perceived by the mouse as different from the normal "sad" stimulus, it could use feedback from rewards to learn essentially a new stimulus identity. Since this new stimulus would be associated with the same response as the normal "sad", it could conceal real effects of the laser. We decided to use random rewards for the main suppression experiments to avoid this possibility, which we observed to some degree in mice during additional testing. While not all mice were able to bring laser trial performance up to control levels, this could be a result of individual variation in learning strategies or the exact amount of suppression in

AC. Mouse 5916 was able to reach indistinguishable performance between the two conditions, showing that this is a real concern. Although it took 15k trials before complete convergence of performance, the trend was developing within several thousand. Additionally, the improvement over time on both control and laser trials in this condition seen in mouse 5916 suggests that the reward type on laser trials may affect overall behavior.

Random rewarding also presents potential problems if there is any way for the mouse to perceive the laser, since it will provide a cue that the response is irrelevant. If the mouse learns this association, it could be disincentivized to perform well on laser trials independent of any impairment of AC activity. This could provide illusory suppression effects that have nothing to do with optogenetic suppression. When the fiber was disconnected but obviously visible to the mouse, we observed this effect occurring within 5k trials in mouse 5982, even though there was no initial effect of the laser in this condition.

Conclusions

Our results support prior work that has identified correlates of speech information maintenance in AC (Engineer 2012, Pasley 2012), providing causal evidence that activity in AC is necessary for phoneme discrimination. The complex spectrotemporal structure of speech involves more advanced processing than relatively simple sounds, requiring the cortical circuitry of AC for accurate perception.

CHAPTER IV: CONCLUSION

Tone Discrimination Summary

In Chapter 2, I examined the relative effects on tone discrimination ability of optogenetic suppression and electrolytic lesions. Optogenetic suppression time-locked to the sound stimulus significantly reduced performance on laser trials, with an average deficit of approximately 15% relative to control trials, while electrolytic lesions had no effect on performance. Although there was variation in the exact extent of lesions in auditory cortex, this was not correlated with the change in performance. Initial experiments (Figures 1 and 2) using optogenetics and lesions had major differences in their temporal structure, with optogenetic suppression trials interleaved within control trials, while lesion analysis was restricted to comparing control trials before the lesion and subsequent post-lesion data. To bridge the gap between these paradigms, I modified the optogenetic experiment structure (Figure 3) by extending suppression to 20 and then 200 trial cycles. In both cycle durations, mice showed a major performance deficit at the beginning of each cycle, with partial subsequent recovery that still remained below control cycle performance. Since there was no way to modify the electrolytic lesion protocol to make it more similar to optogenetics, my final experiment for this chapter (Figure 4) further extended the optogenetic suppression window to 4 hours to match the post-lesion recovery time and provide the most equivalent comparison possible. Comparing performance before and after the extended suppression showed a significant deficit, leading to the conclusion that optogenetic suppression and conventional lesions have fundamentally different effects on the brain.

Phoneme Discrimination Summary

In Chapter 3, I examined the effects of optogenetic suppression on phoneme discrimination and characterized long-term effects of laser-related learning. I observed that behavioral performance decreased when auditory cortex was shut down. This effect was present in 3 different suppression time windows: full stimulus, "consonant", and "vowel" (Figures 1 and 2) and in many cases came in the form of bias toward one side/ response. In addition to the short-term effects of suppression, mice also showed laser-related learning on longer timescales, depending on how trials were rewarded. I tested whether mice could associate the laser with randomly rewarded trials by disconnecting the fiber from their brain and instead leaving it attached to the box where it was clearly visible. In this condition, I observed that mice could learn that their responses on laser trials were irrelevant and stopped attempting to correctly answer on those trials, instead developing a severe bias and showing the importance of minimizing the visibility of the laser during normal experiments. I also tested whether normally rewarding laser trials could allow mice to learn to discriminate between the stimuli even when activity was suppressed. Essentially, I tested whether mice could learn two additional stimuli: "sad" plus laser and "dad" plus laser, and observed that it was possible for some mice, though it took 15,000 or more trials, corresponding to over a month of training. This showed that analysis of optogenetic suppression data should be restricted to early phases of data collection to minimize the chance of confounding effects from laser-related learning.

Remaining Questions for Future Research

While the results presented in this dissertation provide valuable information about the role of auditory cortex in sound perception and discrimination, there are many remaining questions. What compensatory mechanisms are recruited following lesions that remain inactive during optogenetic suppression, and how are they activated? Could alternative stimuli or different timing windows lead to a difference in phoneme discrimination ability that was not present with the 140ms early/late split used in my study? For constant, simple stimuli such as the pure tones used here, what is the relationship between the timing of optogenetic suppression relative to the stimulus and induced deficits in performance? What are the specific task parameters influencing laser-related learning observed in Chapter 3?

Lesions versus Optogenetic Suppression

The combined results of tone discrimination suggest a fundamental difference in the adaptation process following permanent damage or optogenetic suppression. This fits with recent work in several fields, where optogenetic suppression has revealed a role for activity that was obscured by conflicting lesion results (Goshen et al., 2011; Kumar et al., 2013; Otchy et al., 2015; Hong et al., 2018). Electrolytic damage led to rapid and full recovery of behavioral performance, but it is unclear why that was the case. To learn more about what is different in the case of optogenetic suppression, the first step would be to identify the compensatory circuits recruited following lesion damage.

Phoneme Suppression Timing

The relation of suppression timing to sound contents is still poorly understood, as the experiments targeting either the "consonant" or "vowel" component of "sad" and "dad" aren't fully conclusive. Although prior studies have found sufficient information in cortical responses to the earliest parts of phoneme stimuli (Engineer 2008, Centanni 2013), my results showed no difference based on suppression timing. This could be a result of both segments providing relevant information, as due to coarticulation (Ohala 1993), the "ad" components of the two stimuli were not identical and could potentially be sufficient for successful discrimination. Although it would result in stimuli farther from natural speech, one possible experiment to clarify this would involve creating modified chimeric stimuli by copying the "vowel" segment from one recorded stimulus and combining it with the "consonant" segment from another. In this case, the stimuli would only differ in the initial segment, so results showing a greater deficit from targeting the initial segment would suggest that information content is related to the necessity of activity. If instead the results still show equivalent effects of suppression during both segments, interpretation could be difficult. Sustained activity in auditory cortex following the differing "consonant" segments could be required for successful discrimination by facilitating information transfer to downstream areas in the decision making process. If this was the case, suppression during either segment would reduce the information available to areas such as frontal cortex or the striatum.

Pure Tone Suppression Timing

This relationship between suppression timing and behavioral deficits could also be tested using other sound stimuli, such as pure tones. In chapter 2, we only tested suppression over the entire stimulus, but it is possible that the timing could be relevant. If the effects of suppression are only dependent on the information contained within the stimulus, specific timing of suppression during a pure tone should not make a difference, since the sound is constant throughout its duration. Alternatively, if sustained activity is required for transfer of information to downstream areas, it would be possible to identify the precise timing by suppressing activity during the end of the stimulus. Increasing the length of suppression until a deficit is present would indicate the duration of sustained normal activity required for successful discrimination, although this could vary depending on the exact stimulus.

Laser-related Learning

In chapter 3, I examined some potential problems with optogenetic experiments by manipulating laser placement and rewards on laser trials. I observed that mice could learn to associate the light of the laser with random rewards or adapt to suppression by learning via normal rewards on laser trials. This data was anecdotal, however, and came from mice that had prior exposure to suppression in the paradigms of Figures 1 and 2. A focused study on laser-related learning could improve characterization of these two processes. I used either a 10% or 20% proportion of laser trials, but it is likely that this factor influences the development of learning effects. There is a tradeoff between collection of sufficient laser trial data and potential learning that the laser is associated

with random rewards, but it would be valuable to investigate this in detail. I would hypothesize that at some low proportion of laser trials, this type of learning is extremely unlikely, while a proportion that is too high would lead to many mice adjusting their behavior to "give up" on laser trials. Increasing knowledge about this tradeoff would allow researchers to be more informed as they design experiments to balance speed of data collection and potential confounds. Similarly, a higher proportion of laser trials could increase the speed of adaptation to the laser under normal reward conditions.

Visibility of the laser is also likely to be a key factor in the case of associating the laser and random rewards. I attempted to minimize visibility during normal suppression experiments and then dramatically increased it when the fiber was disconnected, but intermediate testing could be valuable. Using a laser of different wavelength that has minimal ability to activate the optogenetic protein would more closely replicate conditions during standard suppression experiments. Equivalently, mice without optogenetic protein expression could be tested with the same implants and laser parameters as transgenic subjects. In chapter 2, I briefly tested wildtype mice in this condition, but did not attempt to characterize long-term effects. From my experiments, it is not totally clear whether normal or random rewards on laser trials is the superior strategy, as normal rewards could obscure real effects of suppression and random rewards could generate illusory effects. I limited analysis to the first 10,000 trials to minimize these confounds based on my observations, but detailed study of these learning processes would improve the design of future experiments.

What Have We Learned?

This dissertation has several key conclusions. First, activity in auditory cortex contributes to maximal pure tone discrimination ability, as revealed by optogenetic suppression experiments. Based on these experiments, optogenetic suppression and electrolytic lesions have fundamentally different effects on the brain, even with the most analogous task parameters. Additionally, optogenetic suppression revealed a similar contribution of auditory cortex activity to phoneme discrimination for the full stimulus as well as during only the early and late segments. Finally, mice can learn laser-based associations when tested for extended periods of time.

REFERENCES CITED

CHAPTER I

Centanni TM, Engineer CT, Kilgard MP. (2013) Cortical speech-evoked response patterns in multiple auditory fields are correlated with behavioral discrimination ability. Journal of Neurophysiology, 110, 177-189. PMCID:PMC3727033

Engineer CT, Perez CA, Chen YH, Carraway RS, Reed AC, Shetake JA, Jakkamsetti V, Chang KQ, Kilgard MP. (2008) Cortical activity patterns predict speech discrimination ability. Nature Neuroscience, 11, 603-608. PMCID:PMC2951886

Gimenez TL, Lorenc M, Jaramillo S (2015) Adaptive categorization of sound frequency does not require the auditory cortex in rats. J Neurophysiol 114:1137–1145.

Helfer, K. S., & Freyman, R. L. (2008). Aging and speech-on-speech masking. Ear and hearing, 29(1), 87–98. doi:10.1097/AUD.0b013e31815d638b

Moore A, Wehr M. (2014) A guide to in vivo single-unit recording from optogenetically identified cortical inhibitory interneurons. J. Vis. Exp. 93, e51757

Ohl F.W., Wetzel W., Wagner T., Rech A., Scheich, H. (1999). Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learning & Memory (Cold Spring Harbor, N.Y.). 6: 347-62.

Porter BA, Rosenthal TR, Ranasinghe KG, Kilgard MP (2011). Discrimination of brief speech sounds is impaired in rats with auditory cortex lesions, Behavioural Brain Research, 219:68-74.

Sakata, S., & Harris, K. D. (2009). Laminar structure of spontaneous and sensory-evoked population activity in auditory cortex. Neuron, 64(3), 404–418. doi:10.1016/j.neuron.2009.09.020

Saunders J. and Wehr M. (2019) Mice can learn phonetic categories. J Acoust Soc Am March 145 (3). doi.org/10.1121/1.5091776

Stosiek C., Garaschuk O., Holthoff K., Konnerth A. (2003) In vivo two-photon calcium imaging of neuronal networks. Proceedings of the National Academy of Sciences. 100 (12) 7319-7324; DOI:10.1073/pnas.1232232100

Talwar SK, Musial PG, Gerstein GL (2001) Role of mammalian auditory cortex in the perception of elementary sound properties. J Neurophysiol 85:2350–2358.

Weible A, Moore A, Liu C, deBlander L, Wu H, Kentros C, Wehr M. (2014) Perceptual Gap Detection is Mediated by Gap Termination Responses in Auditory Cortex. Curr. Bio.24(13):1447-55

CHAPTER II

Aizenberg M, Mwilambwe-Tshilobo L, Briguglio JJ, Natan RG, Geffen MN (2015) Bidirectional Regulation of Innate and Learned Behaviors That Rely on Frequency Discrimination by Cortical Inhibitory Neurons. PLoS Biol 13:e1002308.

Briguglio JJ, Aizenberg M, Balasubramanian V, Geffen MN (2018) Cortical Neural Activity Predicts Sensory Acuity Under Optogenetic Manipulation. J Neurosci 38:2094–2105.

Buser PA, Imbert M (1992) Audition. MIT Press. Butler RA, Diamond IT, Neff WD (1957) Role of auditory cortex in discrimination of changes in frequency. J Neurophysiol 20:108–120.

Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai L-H, Moore CI (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459:663–667.

Gimenez TL, Lorenc M, Jaramillo S (2015) Adaptive categorization of sound frequency does not require the auditory cortex in rats. J Neurophysiol 114:1137–1145.

Goldberg JM, Neff WD (1961) Frequency discrimination after bilateral ablation of cortical auditory areas. J Neurophysiol 24:119–128.

Goshen I, Brodsky M, Prakash R, Wallace J, Gradinaru V, Ramakrishnan C, Deisseroth K (2011) Dynamics of retrieval strategies for remote memories. Cell 147:678–689.

Heffner HE, Heffner RS (1986) Hearing loss in Japanese macaques following bilateral auditory cortex lesions. J Neurophysiol 55:256–271.

Heffner HE, Heffner RS (1990) Effect of bilateral auditory cortex lesions on absolute thresholds in Japanese macaques. J Neurophysiol 64:191–205.

Hong YK, Lacefield CO, Rodgers CC, Bruno RM (2018) Sensation, movement and learning in the absence of barrel cortex. Nature 561:542–546.

Kelly JB (1970) The effects of lateral lemniscal and neocortical lesions on auditory absolute thresholds and frequency difference thresholds of the rat. Diss Abstr Int 31:1566–1567.

Kuchibhotla K, Bathellier B (2018) Neural encoding of sensory and behavioral complexity in the auditory cortex. Curr Opin Neurobiol 52:65–71.

Kumar A, Vlachos I, Aertsen A, Boucsein C (2013) Challenges of understanding brain function by selective modulation of neuronal subpopulations. Trends Neurosci 36:579–586.

Meier P, Flister E, Reinagel P (2011) Collinear features impair visual detection by rats. J Vis 11 74 Available at: http://dx.doi.org/10.1167/11.3.22.

Meyer DR, Woolsey CN (1952) Effects of localized cortical destruction on auditory discriminative conditioning in cat. J Neurophysiol 15:149–162.

Moore AK, Wehr M (2013) Parvalbumin-expressing inhibitory interneurons in auditory cortex are well-tuned for frequency. J Neurosci 33:13713–13723.

Neff WD, Diamond IT, Casseday JH (1975) Behavioral Studies of Auditory Discrimination: Central Nervous System. In: Auditory System: Physiology (CNS)· Behavioral Studies Psychoacoustics (Abeles M, Bredberg G, Butler RA, Casseday JH, Desmedt JE, Diamond IT, Erulkar SD, Evans EF, Goldberg JM, Goldstein MH, Green DM, Hunter-Duvar IM, Jeffress LA, Neff WD, Yost WA, Zwicker E, Keidel WD, Neff WD, eds), pp 307–400. Berlin, Heidelberg: Springer Berlin Heidelberg.

Ohl FW, Wetzel W, Wagner T, Rech A, Scheich H (1999) Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learn Mem 6:347–362.

Otchy TM, Wolff SBE, Rhee JY, Pehlevan C, Kawai R, Kempf A, Gobes SMH, Ölveczky BP (2015) Acute off-target effects of neural circuit manipulations. Nature 528:358–363.

Paxinos G, Franklin KBJ (2019) Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates. Academic Press.

Porter BA, Rosenthal TR, Ranasinghe KG, Kilgard MP (2011) Discrimination of brief speech sounds is impaired in rats with auditory cortex lesions. Behav Brain Res 219:68–74.

Redish DA (2008) MClust Spike Sorting Toolbox.

Rybalko N, Suta D, Nwabueze-Ogbo F, Syka J (2006) Effect of auditory cortex lesions on the discrimination of frequency-modulated tones in rats. Eur J Neurosci 23:1614–1622.

Scholl B, Wehr M (2008) Disruption of balanced cortical excitation and inhibition by acoustic trauma. J Neurophysiol 100:646–656.

Sellick PM (1983) An Introduction to the Physiology of Hearing. By J. O.Pickles.Pp. 341. (Academic Press, London, 1982.) £16.00, 33.00 Hardback. £6.95, 14.50 Paperback. Q J Exp Physiol 68:750–750.

Siegle JH, López AC, Patel YA, Abramov K, Ohayon S, Voigts J (2017) Open Ephys: an open-source, plugin-based platform for multichannel electrophysiology. J Neural Eng 14:045003.

Smith KS, Graybiel AM (2013) A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron 79:361–374.

Talwar SK, Musial PG, Gerstein GL (2001) Role of mammalian auditory cortex in the perception of elementary sound properties. J Neurophysiol 85:2350–2358.

Thompson RF (1960) Function of auditory cortex of cat in frequency discrimination. J Neurophysiol 23:321–334.

Weible AP, Liu C, Niell CM, Wehr M (2014) Auditory cortex is required for fear potentiation of gap detection. J Neurosci 34:15437–15445.

CHAPTER III

Centanni, T. M., Engineer, C. T., and Kilgard, M. P. (2013). Cortical speech-evoked response patterns in multiple auditory fields are correlated with behavioral discrimination ability. Journal of Neurophysiology 110, 177–189. doi:10.1152/jn.00092.2013.

Centanni, T. M., Sloan, A. M., Reed, A. C., Engineer, C. T., Rennaker, R. L., 2nd, and Kilgard, M. P. (2014). Detection and identification of speech sounds using cortical activity patterns. Neuroscience 258, 292–306.

Chang, E. F., Rieger, J. W., Johnson, K., Berger, M. S., Barbaro, N. M., and Knight, R. T. (2010). Categorical speech representation in human superior temporal gyrus. Nat. Neurosci. 13, 1428–1432.

Cranford, J. L., Igarashi, M., and Stramler, J. H. (1976). Effect of auditory neocortex ablation on pitch perception in the cat. J. Neurophysiol. 39, 143–152.

DeisserothLab Predicted irradiance values: model based on direct measurements in mammalian brain tissue. Brain tissue light transmission calculator. Available at: https://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php [Accessed September 1, 2019].

Engineer, C. T., Centanni, T. M., and Kilgard, M. P. (2016). Rodent Models of Speech Sound Processing. Neurobiology of Language, 829–838. doi:10.1016/b978-0-12-407794-2.00066-3.

Engineer, C. T., Perez, C. A., Chen, Y. H., Carraway, R. S., Reed, A. C., Shetake, J. A., et al. (2008). Cortical activity patterns predict speech discrimination ability. Nat. Neurosci. 11, 603–608.

Flinker, A., Chang, E. F., Barbaro, N. M., Berger, M. S., and Knight, R. T. (2011). Sub-centimeter language organization in the human temporal lobe. Brain Lang. 117, 103–109.

Floody, O. R., Ouda, L., Porter, B. A., and Kilgard, M. P. (2010). Effects of damage to auditory cortex on the discrimination of speech sounds by rats. Physiology & Behavior 101, 260–268. doi:10.1016/j.physbeh.2010.05.009.

Green, D. M., and Swets, J. A. (1966). Signal detection theory and psychophysics. Wiley New York.

Johnson, K. L., Nicol, T. G., and Kraus, N. (2005). Brain stem response to speech: a biological marker of auditory processing. Ear Hear. 26, 424–434.

Kilgard, M. P., and Engineer, C. T. (2015). Neural Coding of Speech Sounds. Encyclopedia of Computational Neuroscience, 1–4. doi:10.1007/978-1-4614-7320-6_433-2.

Liebenthal, E., Binder, J. R., Spitzer, S. M., Possing, E. T., and Medler, D. A. (2005). Neural Substrates of Phonemic Perception. Cerebral Cortex 15, 1621–1631. doi:10.1093/cercor/bhi040.

Meier, P., Flister, E., and Reinagel, P. (2011). Collinear features impair visual detection by rats. J. Vis. 11. doi:10.1167/11.3.22.

Moore, A. K., and Wehr, M. (2013). Parvalbumin-expressing inhibitory interneurons in auditory cortex are well-tuned for frequency. J. Neurosci. 33, 13713–13723.

Ohl, F. W., Wetzel, W., Wagner, T., Rech, A., and Scheich, H. (1999). Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learn. Mem. 6, 347–362.

Pasley, B. N., David, S. V., Mesgarani, N., Flinker, A., Shamma, S. A., Crone, N. E., et al. (2012). Reconstructing speech from human auditory cortex. PLoS Biol. 10, e1001251.

Perez, C. A., Engineer, C. T., Jakkamsetti, V., Carraway, R. S., Perry, M. S., and Kilgard, M. P. (2013). Different timescales for the neural coding of consonant and vowel sounds. Cereb. Cortex 23, 670–683.

Porter, B. A., Rosenthal, T. R., Ranasinghe, K. G., and Kilgard, M. P. (2011). Discrimination of brief speech sounds is impaired in rats with auditory cortex lesions. Behav. Brain Res. 219, 68–74.

Redish, D. A. (2008). MClust Spike Sorting Toolbox.

Saunders, J. L., and Wehr, M. (2019). Mice can learn phonetic categories. J. Acoust. Soc. Am. 145, 1168.

Schnupp, J. W. H., Hall, T. M., Kokelaar, R. F., and Ahmed, B. (2006). Plasticity of temporal pattern codes for vocalization stimuli in primary auditory cortex. J. Neurosci. 26, 4785–4795.

Shetake, J. A., Wolf, J. T., Cheung, R. J., Engineer, C. T., Ram, S. K., and Kilgard, M. P. (2011). Cortical activity patterns predict robust speech discrimination ability in noise. Eur. J. Neurosci. 34, 1823–1838.

Siegle, J. H., López, A. C., Patel, Y. A., Abramov, K., Ohayon, S., and Voigts, J. (2017). Open Ephys: an open-source, plugin-based platform for multichannel electrophysiology. J. Neural Eng. 14, 045003.

Tsunada, J., Lee, J. H., and Cohen, Y. E. (2011). Representation of speech categories in the primate auditory cortex. J. Neurophysiol. 105, 2634–2646.

Weible, A. P., Liu, C., Niell, C. M., and Wehr, M. (2014). Auditory cortex is required for fear potentiation of gap detection. J. Neurosci. 34, 15437–15445.

CHAPTER IV

Centanni TM, Engineer CT, Kilgard MP. (2013) Cortical speech-evoked response patterns in multiple auditory fields are correlated with behavioral discrimination ability. Journal of Neurophysiology, 110, 177-189. PMCID:PMC3727033

Engineer CT, Perez CA, Chen YH, Carraway RS, Reed AC, Shetake JA, Jakkamsetti V, Chang KQ, Kilgard MP. (2008) Cortical activity patterns predict speech discrimination ability. Nature Neuroscience, 11, 603-608. PMCID:PMC2951886

Goshen I, Brodsky M, Prakash R, Wallace J, Gradinaru V, Ramakrishnan C, Deisseroth K (2011) Dynamics of retrieval strategies for remote memories. Cell 147:678–689.

Hong YK, Lacefield CO, Rodgers CC, Bruno RM (2018) Sensation, movement and learning in the absence of barrel cortex. Nature 561:542–546.

Kumar A, Vlachos I, Aertsen A, Boucsein C (2013) Challenges of understanding brain function by selective modulation of neuronal subpopulations. Trends Neurosci 36:579–586.

Ohala, John J. (1993). Coarticulation and phonology. Language and Speech, vol. 36, 2-3: pp. 155-170.

Otchy TM, Wolff SBE, Rhee JY, Pehlevan C, Kawai R, Kempf A, Gobes SMH, Ölveczky BP (2015) Acute off-target effects of neural circuit manipulations. Nature 528:358–363.